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Hybridization and conservation of tilapia cichlid fish biodiversity in Tanzania

HYBRIDIZATION AND CONSERVATION OF TILAPIA CICHLID FISH BIODIVERSITY IN TANZANIA.

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A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Masters by Research in the Faculty of Science.

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Student Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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Thesis Abstract:

Freshwater habitats are the most threatened on the planet, facing impacts from overfishing, habitat degradation, and the introduction of non-native invasive species. This thesis focuses on the importance of freshwater ecosystems for fisheries and aquaculture, and the threats that are currently facing the unique biodiversity of these systems. In Chapter 1 I discuss the importance of freshwater ecosystems, and review the key threats faced by freshwater fish species. I focus on the Nile Tilapia (*Oreochromis niloticus*), a species that has been widely introduced to non-native habitats for capture fisheries improvement, and following escapes from aquaculture ponds. I describe how this invasive species can impact on unique genetic resources of native species through hybridization. In Chapter 2 I report an investigation of hybridization between *Oreochromis niloticus* and newly discovered populations of an indigenous species, *Oreochromis korogwe*, that were first reported from southern Tanzania in 2013. Using genetic (microsatellite) evidence, I show that hybridization is taking place in all three locations in southern Tanzania where *O. korogwe* is known to occur (Lakes Nambawala, Rutamba and Mitupa). I also show that the *O. korogwe* in the southern Tanzania are genetically and morphologically different to populations known from northern Tanzania. I conclude that these newly discovered southern Tanzanian populations are already being threatened by hybridization with an invasive species that threatens their unique and irreplaceable genetic resources. In Chapter 3 I highlight the research requirements needed to further understand the extent of hybridization between indigenous and non-native fish species in Africa, and to inform future conservation and management plans.

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Contents

Student Declaration I
Thesis abstract II
Acknowledgements III
Table of Contents IV
List of Tables V
List of Tables VI
List of Figures VII
List of Appendices VIII
Acronyms IX

Chapter One
1.1 Importance of Freshwater Fisheries 1
1.1.1 Ecosystem Services 2
1.1.2 Jobs 3
1.1.3 Food Security 3
1.1.4 Biodiversity 4
1.2 Conservation of Freshwater Fishes and Fisheries 5
1.2.1 Inland Fisheries Sustainability 5
1.2.2 Habitat Degradation 6
1.2.3 Invasive Species 10
1.3 Hybridization 15
1.3.1 Impacts of hybridization 15
1.3.2 Occurrence of hybridization 19
1.3.3 Methods used to identify hybrid individuals 22
1.4 The Project 24

Chapter Two
2.1 Abstract 28
2.2 Introduction 29
2.3 Methods 31
2.4 Results 38
2.5 Discussion 45

Chapter Three
3.1 Research Summary 50
References 61

List of Tables:

Table 1. Sample sizes for southern comparison analysis. 32

Table 2. Sample sizes for comparisons of southern and northern populations.....32

Table 3. Original and predicted group membership results for southern morphological analysis. 39

Table 4. Correlation of traits with Discriminant Function Axis 1 separating *O. niloticus* from *O. korogwe* in the southern lakes 39

Table 5. Matrix of F_{ST} pairwise comparisons (below left) and corresponding *P* values from Exact tests (above right).....42

Table 6. Morphological differences among southern and northern populations of *O. korogwe*, tested using Discriminant Function Analysis. 43

Table 7. Classification results from Discriminant Function Analysis of four populations of *O. korogwe* from a) traditional measures of morphology and b) geometric morphometric measures. 44

Table 8. Correlation of traits with Discriminant Function axes separating *O. korogwe* populations, using linear (traditional) measurements. 45

List of Images:

Image 1. Major watersheds of Tanzania and distribution of *Oreochromis* species. 14

Image 2. Distributions of native *Oreochromis spp*. across Tanzania.....25

List of Figures:

Figure 1. Sampling locations of focal sites across Tanzania.....33

Figure 2. Photographs of Specimens used.....34

Figure 3. Genetic analysis of southern populations.....38

Figure 4. Morphological analysis of southern populations.....40

Figure 5. Genetic analysis of populations of northern and southern populations.....41

Figure 6. Canonical variate analysis of northern and southern *O. korogwe* 43

Figure 7. Discriminant Analysis of morphology of *northern and southern O. korogwe*.....44

List of Appendices

Supplementary Information Figure S1. Landmarks used in the geometric morphometric analysis. 54

Supplementary Information Table S1. Genetic diversity of the focal populations of *O. korogwe*, *O. urolepis* and *O. placidus*. 55

Supplementary Information Table S2. Morphological variation of focal populations of *O. korogwe*, *O. niloticus* and *ON*x*OK* hybrids 56

Supporting Information Table S3: Microsatellite loci primer sequences and sources. 57

Supporting Information Table S4: Allele frequencies per population 58

Acronyms

CVA: Canonical Variates Analysis

DFA: Discriminant Function Analysis

FAO: Food and Agriculture Organization of the United Nations

FCA: Factorial Correspondence Analysis

FPA: Freshwater Protected Areas

GDP: Gross Domestic Product

GVA: Gross Value Added

IUCN: Union for Conservation of Nature

MEA: Millennium Ecosystem Assessment

OK: *Oreochromis korogwe*

ON: *Oreochromis niloticus*

PCR: Polymerase Chain Reaction

SDA: Small Dam Density

UN: United Nations

P-C: *O. placidus* Lake Chidya

U-R: *O. urolepis* Rufiji river

K-Z: *O. korogwe* Zigi river

K-R: *O. korogwe* Lake Rutamba

K-N: *O. korogwe* Lake Nambawala

Chapter 1: Literature review.

Importance of freshwater fisheries and aquaculture

Around 71% of the world's surface is covered by water, and less than 3% of that is freshwater, yet it provides a substantial range of ecosystem services, both locally and globally. The Food and Agriculture Organization of the United Nations (FAO, 2016) estimated that global inland capture fisheries production rose from 10.5 million tonnes in 2009 to almost 12 million tonnes in 2014 (FAO, 2016). An estimated 80% of this global inland capture production occurs in just 16 countries, with the catches of these countries reported as exceeding 200,000 tonnes each annually. Importantly, approximately 90% of inland fisheries catches come from developing countries (FAO, 2016). Despite the productivity of fisheries globally, inland fisheries are often overlooked in decision and policy making (McIntyre *et al.* 2016). This often occurs due to a lack of available data on stocks, productivity, and uses of inland fisheries (McIntyre *et al.* 2016). In fact, of 218 countries, only 98 provided information at all on inland freshwaters for the global fisheries assessment, leading to some large gaps in our knowledge of fish stocks worldwide (FAO, 2016).

It is thought that inland fisheries production may even be higher than that of marine fisheries in some areas (Welcomme *et al.* 2010; Bartley *et al.* 2015; Deines *et al.* 2017), but the absence of data prevents full assessments to be made. Since the majority of inland fisheries production occurs in developing countries, a large amount of fish production does not enter the monitored economy and is sold locally or consumed close to the point of capture. This local use of inland resources is another reason inland fisheries production is often undervalued, and under-estimated both in production and services value. Another important consideration is the scattered and often remote nature of the inland fisheries, that are difficult to access and assess (Deines *et al.* 2017; Welcomme *et al.* 2010). Filling in these gaps in our knowledge will be essential to inform policy and decision makers of the true importance of inland fisheries in the production of a variety of often irreplaceable ecosystem services.

Production in the aquaculture sector reportedly overtook production of wild fish for the first time in 2014 (FAO, 2016). Total aquaculture production has risen from 55.7 million tonnes in 2009, to 74 million tonnes in 2014. Of this, inland aquaculture production has risen from 34.3 million tonnes in 2009 to 47 million tonnes in 2014 (FAO, 2016). The first sale value of fish and aquatic animal production in 2014 was estimated at US$160.2 billion, which accounts for more than 60% by value of total global production of fish that year (FAO, 2016). Aquaculture is now one of the fastest growing

food production sectors globally. The most common operation of aquaculture is that of inland finfish culture, which has shown an increase in production of 5.8% annually, resulting in a 65% global increase between 2005 and 2014 (FAO, 2016). There are 35 reported countries, covering 45% of the world's population, that produce more fish from farming than from wild-caught practices when measured nationally. These include China, India, Vietnam, Bangladesh and Egypt (FAO, 2016).

In Africa, the value added by the fisheries and aquaculture sector was reported by the FAO as around US$24.0 billion, which covers 1.26% of the Gross Domestic Product (GDP) of all African countries combined in 2011 (FAO, 2016). In total African inland fisheries provided a Gross Value (GVA) of US$6275 million, which is 0.33% of the GDP of all African countries. Marine artisanal fisheries provided a larger GVA of more than US$8,130 million (0.43%), and marine industrial fisheries provided US$6849 million (0.36%). Aquaculture provided 0.15% of the GDP of Africa, with US$2,776 million in production in 2011 (FAO, 2016). Fishing activities vary regionally across the continent, with West Africa dominated by artisanal fisheries, and Southern Africa mostly by industrial fisheries. In Central Africa, inland fisheries make up the majority of the sector (FAO, 2016). In Tanzania, inland fishing provides US$836 million, while marine artisanal fishing provides US $146 million and has a total Gross Production Value of US$982 million.

Despite the growth of the aquaculture industry globally, in Africa, development has been relatively slow, constrained by a lack of funding for effective management and the implementation of new technology (Lind *et al.* 2012a). Finfish production from aquaculture across Africa was reported at 1.7 million tonnes in 2014, and 7,240 tonnes of crustaceans in the same year (FAO, 2016). While aquaculture for consumption is a relatively young sector, its purpose for recreational use is much older. The recreational sector still has a large importance for some African economies, despite its seemingly low fisheries production (Ellender *et al*. 2014).

Ecosystem services of freshwaters

Freshwaters provide multiple ecosystem-scale goods and services, that include the provision of drinking water, irrigation water for agriculture, hydropower, recreation, and food (Deines *et al.* 2017). These services can create conflicts for the use of freshwater resources, and alongside growing population sizes, are putting increasing pressures on freshwater systems (Dudgeon *et al.* 2006). Alongside production of fish for human consumption, inland fisheries have an important contribution to recreational uses, estimated to involve more than 100 million people in North America, Oceania,

and Europe. In the United States alone, recreational fishing is valued at over US$40 billion (FAO, 2016).

Jobs. Globally, 56.6 million people were reported to work in the capture fisheries and aquaculture sector in 2014, and 84% of these were in Asia, followed by 10% in Africa (FAO, 2016). Recent estimations show more than 60 million people working either directly or indirectly within the inland capture fisheries sector, with women representing more than half (FAO, 2016). It is estimated that total employment across the whole sector in Africa includes 25.4 million people, with fishing accounting for 7.8 million, and 17.6 million in post-harvest processing (FAO, 2016). Almost 2 million people are employed in the inland fisheries sector alone, with 66% fishermen. 26% of the people employed in inland fisheries are women, and the majority work as processors (FAO, 2016). For example, in Tanzanian inland fisheries 207,787 male employees are fishers, with 3,543 female fishers. Male processors make up 111,100, while female processors total 123,551. The inland fishery total employment was 445,981 people for Tanzania in 2011 (FAO, 2016).

Food security. This is defined as having three main aspects; availability, accessibility, and stability (Hishamunda and Ridler, 2006). Despite continued effort to alleviate malnutrition, starvation, and poverty, there remains over 795 million people reported to be undernourished globally (Ahmed and Lorica, 2002; FAO, 2016). Faced with this concern over the state of malnutrition, the International Conference of Nutrition has highlighted the growing importance of the fisheries and aquaculture sectors in providing a viable solution (FAO, 2016). Globally, it is estimated that more than 30% of the world's population relies on fish and fish products for 20% of their animal protein (FAO, 2016). Given that most freshwater fisheries are in developing countries, it is unsurprising that 81% of dependence on freshwater fisheries occurs in nations with below global median GDP (McIntyre *et al.* 2016). In these Low-Income-Food-Deficit countries, fish provide 3.5 - 7.6 kg per capita for consumption (FAO, 2016). It therefore follows that reductions in inland fisheries production, through over-harvesting and other anthropogenic factors, are likely to have the largest impact on poorer, more vulnerable populations due to their inability to access alternative sources of food security or livelihoods (Lynch *et al.* 2017). Thus, sustainable inland fisheries play a vital role in securing the United Nations (UN) 2030 Agenda for Sustainable Development (Lynch *et al.* 2017).

Aquaculture has been one of the fastest growing food-producing sectors globally, and therefore has been suggested as a potential for increasing food security, particularly in developing countries (Ahmed and Lorica, 2002). Aquaculture is fast becoming recognised as a generator of good income

for rural households, and therefore has the potential to hold fish prices down, make fish a more accessible resource, increase consumption and therefore benefit nutrition (Ahmed and Lorica, 2002). However, it may not be a completely viable option for alleviating poverty, as it often excludes poorer sectors of society (Lynch *et al.* 2017). In cases where cultured species require feed that must be harvested from wild populations, there is no alleviation of wild fisheries pressure, and often takes resources that would usually be consumed by poorer communities for use in feed (Lynch *et al.* 2017).

Biodiversity. Biodiversity is defined as the variation in the natural world at the level of genes, populations, species, and ecosystems, and is essential for the proper functioning of all ecosystems on earth. It is an integral part of all life on earth, and yet knowledge there are many gaps in our knowledge of the extent of biodiversity on the planet, and the extent to which it is threatened. The Millennium Ecosystem Assessment was established to fill in these gaps in our knowledge of global biodiversity, and the goods and services they provide, both for use in the management of ecosystems for human use, and to provide a comprehensive framework to assess the need for conservation efforts (MEA, 2016).

A lot of the work assessing biodiversity globally has focused on terrestrial systems, and much more is understood about the impact of biodiversity on the quantity and quality of the services such terrestrial environments provide. Much less is known about freshwater ecosystems, despite their significant contribution to global biodiversity (Mantel *et al.* 2017). There is growing evidence to suggest that the services provided by freshwater ecosystems could be impacted by changes in biodiversity (Dudgeon *et al.* 2006). Ecological theory also suggests that biodiversity is crucial to ecosystem productivity (McIntyre *et al.* 2016). Research has also shown that freshwater fish species richness is highly correlated with yield and is an important predictor of yield once other factors are accounted for (Brooks *et al.* 2016). Freshwater ecosystems provide a range of direct and indirect goods and services, with many being irreplaceable (Pearce, 1998), and are likely to be impacted by the loss of biodiversity. The first estimate of the global value provided by all inland waters yielded a value of US$6579 x 10^9 per year (Costanza *et al.* 1997). There has been some conflict over the exact value, but many estimates fall at similar values (Dudgeon *et al*. 2006). The valuation of ecosystem services has often been used to help lead policy decisions, and to facilitate the evaluation of conservation priorities, as well as to provide clear justification for the public spending on conservation efforts (Turpie *et al.* 2017). This justification allows a change in public attitudes toward conservation and plays a large role in the success of such objectives.

Biodiversity is also a crucial source of genetic resources, which are essential for the long-term viability of the fisheries industry, especially for use in aquaculture or to re-stock wild populations (Soler *et al.* 2010; Lind *et al.* 2012a). Cultured species have often been shown to have reduced genetic variation over successive generations (Lind *et al.* 2012a; Lind *et al.* 2012b), therefore stocking with wild resources may contribute to the long-term viability of the sector. Genetic resources are also an important tool for selecting advantageous traits to increase aquaculture production, for example male sex determination and fast growth rates (Bentsen *et al.* 2017; Ndiwa *et al.* 2014; Bernatchez *et al.* 2017). Aquaculture escapes have often been accused of impacting freshwater biodiversity through introductions into wild populations (Conte *et al.* 2017; Lind *et al.* 2012a), therefore careful management schemes need to be in place to ensure the sustainability of these practices that are so crucial for the global economy.

Conservation of Freshwater Fishes and Fisheries

Inland Fisheries Sustainability. Given the key role of marine and inland capture fisheries and aquaculture in providing over 3 billion people with essential animal proteins, it is crucial to manage our global stocks sustainably. Over-harvesting of marine fisheries, causing declines and even collapses of several industries has been receiving increasing attention in recent years, yet there has been very little attention given to inland fisheries (Allan *et al.* 2005). Freshwater systems are understood to be one of the most diverse ecosystems in the world, and yet often the most threatened by human use (Arroita *et al.* 2017). Recent estimates have suggested that 31.4% of fish stocks are considered overfished, and this number is probably an underestimate as it does not include the 80% of fish stocks that remain un-assessed (Allan *et al.* 2005; FAO, 2016). Over-harvesting is often known to pre-date other anthropogenic impacts on fishery declines, and there are many historic examples of inland fisheries being harvested well above sustainable and profitable levels (Allan *et al.* 2005). For example, Murray cod (*Maccullochella peelii*) in the Murray-Darling river in Australia was harvested to a level far beyond that of commercial profitability, and while there has been some recovery when this level of harvesting was reduced, other anthropogenic pressures have now replaced this issue in damaging this fishery (Allan *et al*. 2005). There have also been many contemporary examples, including that of the Nile perch (*Lates niloticus*) in Lake Victoria (Allan *et al.* 2005). The loss of apex predators is known to have top-down effects on the community structure of freshwater ecosystems and often leads to a reduction in ecosystem functioning and production (Allan *et al.* 2005).

The historic, and contemporary, issues of over-harvesting fisheries and the resulting collapse in various industries, have led to an increased attention on the 'sustainability' of these sectors (Allan *et al.* 2005). In fisheries, sustainability is often measured as the stock of the particular species of interest, as well as the mortality or fishing pressure on that specific species (Kolding and van Zwieten, 2012). Fishing patterns therefore focused on taking only adult individuals, leading to a size selective harvesting pattern (Kolding and van Zwieten, 2012). Research has shown the potential damages to this process, such as the disruption of trophic and demographic structuring, and early maturation and reduced adult body size of target fish, which may unintentionally reduce potential yields (Kolding and van Zwieten 2012; McIntyre *et al.* 2016). As well as reducing yields, in the long-term, these fishing methods could fundamentally alter the functioning of freshwater ecosystem processes (McIntyre *et al.* 2016).

Fisheries research is now focusing more on an ecosystem approach, which takes into account the structure and functioning of the whole ecosystem, rather than the status of one species (Kolding and van Zwieten, 2012). Efforts are being made to change the methods used in fisheries to produce a more balanced harvesting system. These efforts suggest that a balance of different fishing methods to harvest fish across the whole community, and across a range of sizes, could produce higher yields whilst protecting biodiversity (Garcia *et al.* 2012). An example of this type of harvesting can be seen at Lake Dolta, a reservoir of around 8500 km^2 in Ghana (Garcia *et al.* 2012). Over many years, fishers have produced a system of using a variety of around 27 different fishing systems to harvest a range of available resources within the lake. Estimates show that this lake produces 250,000 tonnes annually using this system (Garcia *et al.* 2012). The lake undergoes fluctuating water levels due to seasonal flooding, and fishers vary their fishing method and efforts accordingly, as well as varying their harvest over different habitats throughout the year (Garcia *et al.* 2012). The different fishing gear targets fish of differing lengths, allowing fishers to act like niche-partitioned predators targeting specific prey (Garcia *et al.* 2012). By varying this selective pressure over the course of the year they are able to target all the available resources and thus provide high yields at minimal cost to the functioning of the ecosystem (Kolding and van Zwieten, 2012).

Habitat degradation: Habitat degradation can occur from multiple (often interacting) factors which either directly or indirectly impact the environment. Human land use is often to blame for these impacts, for example, forest clearance for urban expansion or cropland, often leads to sediment run-off, which can cause shoreline erosion and sometimes even flooding (Dudgeon *et al.* 2006).

Freshwater ecosystems are particularly threatened by the demands of humans for water, and there is considerable, and ever-increasing, competition among stakeholders (Dudgeon *et al.* 2006). There are 263 international rivers that are responsible for draining 45% of the Earth's surface, and this area supports more than 40% of the global human population showing the immediate human demands on these systems (Dudgeon *et al.* 2006). Over half of the world's rivers are affected by dams (Nilsson *et al*. 2005), and it has been suggested that there no longer remains a substantial number of water bodies that are not irreversibly altered by human activities (Dudgeon *et al.* 2006). In fact, there are now measurable geo-dynamic changes in the Earth's rotation and gravitational field due to the creation of dams across the Northern Hemisphere (Chao, 1995; Dudgeon *et al.* 2006). In the 2016 FAO report, it was estimated that around 10% of the world's freshwaters were abstracted annually for human use (FAO, 2016). More than 70% of this is attributable to agriculture, 20% to industry and a further 10% to domestic use (FAO, 2016). These figures demonstrate the immense and often conflicting pressures put upon these systems (Dudgeon *et al.* 2006).

In Africa, habitat degradation is having major impacts for freshwater ecosystems, due to expanding deforestation, fishing pressures, and rising water temperatures (Cohen *et al.* 2016). The African Great lakes represent some of the most biodiverse freshwater ecosystems on Earth, for example Lake Tanganyika supports over 1470 animal species, including over 200 cichlid species, 97% of which are endemics (Cohen *et al.* 2016; Britton *et al.* 2017;). This lake is being heavily impacted by human disturbance, with only 6% of its shoreline protected (Britton *et al.* 2017). One of the most damaging impacts is that of sedimentation from watershed deforestation, which is negatively impacting habitat quality, heterogeneity, and species community functioning (McIntyre *et al.* 2005; Britton *et al.* 2017). Urban and industrial waste introduction is also increasing (Britton *et al.* 2017) and impacting the physiology of major fish species leading to reduced fitness (Napit, 2013). There are also many indirect effects that need further study, for example in Lake Victoria, cichlid visual communication is being indirectly impacted by reducing water clarity, reducing the diversity of species (Seehausen *et al.* 1997). These impacts are widespread, and protected areas are often created with little consideration for conservation of freshwater biodiversity (Britton *et al*. 2017).

Habitat degradation, along with invasive species, is one of the major causes of species decline (Hermoso *et al.* 2011). This is particularly the case in freshwater ecosystems because their patchy distribution within the terrestrial and marine landscape elevates their vulnerability (Dudgeon *et al.* 2006; Bush and Hoskins, 2017). Future land use changes are predicted to include the expansion of

urbanisation and rising crop commodity prices, driven by with rising human population sizes and increased demands for living space and food (Martinuzzi *et al.* 2014). Agricultural practices have several impacts on the freshwater environment through sediment build-up, pesticide and other nutrient run-off entering rivers (Meyer *et al.* 1988; Martinuzzi *et al*. 2014).

Water abstraction for irrigation and for dam construction have various hydrological impacts, as they modify the natural flow of water (Olden and Naiman, 2010). These practices also reduce the quantity and quality of riparian habitats, impacting both freshwater and terrestrial fauna and often reducing freshwater biodiversity (Arroita *et al.* 2017; Poff *et al.* 2003; Dudgeon *et al.* 2006). Habitat degradation has been shown to alter interspecific interactions and therefore the ecology of a variety of species, although the full extent of this is not yet fully understood (McIntyre *et al.* 2005).

Dams are often constructed with little thought for the longitudinal and latitudinal movement of freshwater fishes, and so obstruct their migration and put stress on these fish in different parts of their habitats at different times (Dudgeon *et al.* 2006). Species with restricted ranges are particularly threatened, for example more than half of freshwater fish species in America are range restricted and are projected to have between 3 to 44% of their distribution affected by future land use changes (Januchowski-Hartley *et al.* 2016). The impact of these habitat alterations is of particular concern given their vulnerability to the interacting impact of climate change, i.e. dams constrict species from altering their distributions in response to climate change (Kano *et al.* 2016).

Given the level of human dependence on freshwater ecosystems, and the reality of this dependence continuing to grow, there has been growing recognition that new, and more effective, management actions will need to be implemented (Poff *et al.* 2003; Olden and Naiman, 2010). Recently, a new paradigm for the management of these activities is emerging as new partnerships between stakeholders are formed to develop approaches to whole-river management (Poff *et al.* 2003). 'Reconciliation ecology' is often the term used for this new approach, which aims to balance the need to conserve freshwater biodiversity with human needs for water in a realistic manner (Dudgeon *et al.* 2006). The link between biodiversity and fishery yields provides a clear human incentive for conservation (Brooks *et al*. 2016).

It is generally accepted that regulation of water flow in freshwater systems needs to reflect a natural flow variability to maintain normal ecosystem functioning (Olden and Naiman, 2010). A key question to answer is exactly how much flow regulation is needed, and what level of natural flow can be

conceived as sustainable in the long term (Poff *et al.* 2003). Incorporating 'Environmental Flows' into riverine management has been suggested as a new approach to balancing these conflicts with biodiversity demands (Olden and Naiman, 2010). However, these management schemes have so far focused on restoring water quantity, and often ignore the importance of water quality, that is often degraded by human activities (Olden and Naiman, 2010).

Research has suggested that there is a potential for policy changes and market factors to influence future land use change and thus freshwater systems. For example, in the USA two scenarios were proposed to reduce cropland cover and urban expansion termed "Forest Incentives" and "Urban Containment" respectively (Martinuzzi *et al.* 2014). These incentives, while not always effective, saw some reductions in these activities in some areas of the country, and could be a potential first step to align human attitudes with conservation efforts in freshwaters (Martinuzzi *et al.* 2014). Creating scenarios of future land use changes to inform decision and policy makers has been especially useful when used with information on species distributions as it allows an analysis of which species will be impacted by future plans, and which species should be prioritised for conservation, regardless of their current IUCN listing (Januchowski-Hartley *et al*. 2016). Longer term goals need to be considered, especially when taking into account climate change alongside future land use changes (Bush *et al.* 2014).

One of the most popular methods to solving issues of fragmentation in freshwater ecosystems is the removal of barriers to restore tributary connectivity (Neeson *et al.* 2015). Management and funding of these projects occurs at a variety of spatial and temporal scales, but research has suggested that the most successful projects are those that are co-ordinated across time and space, over much longer time scales (Neeson *et al.* 2015). Smaller projects benefit from large-scale prioritisation, so that they allow connectivity between projects, therefore more effectively mitigating for the impacts of fragmentation (Neeson *et al.* 2017). There is some evidence that some species can recover after the removal of dams (Mantel *et al.* 2017), but often habitat quality cannot recover in the same way, and species richness is often drastically reduced (Lehosmaa *et al.* 2017).

Another conservation strategy is the creation of Freshwater Protected Areas (FPAs), which aim to limit the anthropogenic disturbance in areas designated as priority areas for conservation (Britton *et al.* 2017). Limiting the future construction of dams in these areas is crucial to managing the level of human disturbance to freshwater ecosystems (Kano *et al*. 2017). Terrestrial-focused protected areas have been shown to be useful in protecting freshwater biodiversity, through the reduction in sediments

and pollutants entering the water (Britton *et al.* 2017). However, more research is needed to investigate the reaches of these positive effects beyond the borders of the protected areas (Britton *et al.* 2017), and whether other factors need to be considered in designing future protected areas, such as the interacting impact of climate change (Cohen *et al.* 2016).

To manage impacts of altering freshwater habitats there is a need to identify the early and initial signs of biodiversity impairment (Colas *et al.* 2016). There have been a variety of methods used to assess the extent of habitat degradation, including biological indicators such a fungus species richness, which has been successfully used to measure the integrity of freshwater reservoirs after dam construction (Colas *et al.* 2016). One physical threshold indicator has been put in place to monitor habitat degradation is the density of small dams, termed the Small Dams Density (SDD) index (Mantel *et al.* 2017), which allows management to meet the UN Sustainable Development Goals for a sustainable future (Mantel *et al.* 2017).

It has been suggested that a variety of methods will be needed for management efforts to be effective, and perhaps a more realistic approach would be to accept that realistically there is no 'win-win' situation in which the world's water can be completely protected, while also continuing to provide all the goods and services necessary to sustain the ever-growing human population (Strayer and Dudgeon, 2010).

Invasive species. Introductions of species to new areas has been occurring both intentionally and accidentally for thousands of years. Invasive alien species are defined as species that are introduced and have become established in areas outside their normal distributions, often impacting native species and causing economic or environmental damage. Invasive species are considered, alongside climate change, to be one of the drivers of biodiversity change that is hardest to reverse (MEA, 2016). In freshwater ecosystems, invasive alien species are the second leading cause of species extinctions (Dudgeon *et al.* 2006; MEA, 2016).

Humans have historically introduced these species accidentally through practices such as pet trade, escapes from aquaculture or during the movement of traded goods (Marr *et al.* 2010). Intentional introductions have occurred often for use in hunting, for ornaments or pets, or for use as biological control (Marr *et al.* 2010). Introductions of non-native species also occur without the intervention of humans, for example through biogeographic changes such as lake or sea level fluctuations or temperature shifts allowing large scale range expansions, or accidentally through the movement of

other species (Olden *et al.* 2010). These non-human mediated introductions do not occur often in freshwater environments, due to the obvious dispersal constraints of surrounding land (Olden *et al.* 2010). Therefore, without human intervention, expansions mainly occur due to very rare events, including flooding, or river captures (Olden *et al*. 2010).

Across Africa, non-native species have been intentionally introduced for multiple uses, often with little regard or concern for the long-term consequences (Ellender *et al.* 2014). Stock enhancements have been encouraged by local governments, for use in recreational angling, and later for commercial fisheries and aquaculture (Skelton and Davies, 1986; McCafferty *et al.* 2012; Ellender *et al.* 2014). Non-native species have established many commercial and recreational fisheries, and therefore their complete eradication is not always beneficial (Ellender *et al.* 2012; Ellender and Weyl, 2014). For example, despite the rich diversity of freshwater fish species across Africa (3,000 species across 483 genera; Skelton and Davies, 1986), the aquaculture sector is still in its infancy and depends on introduced species for a large proportion of production (Brummett and Williams, 2000). It was not until the late 1960s that the negative impacts of these introduced species became impossible to ignore, and public opinion is starting to change in favour of preventing continued introductions of these species across Africa (Ellender *et al.* 2014).

Species are likely to become invasive when they show high growth rates, high fecundity and are readily adaptable to a variety of habitats (Ruesink, 2005; Olden *et al.* 2010; Agdamar *et al.* 2015). These attributes allow them to establish in new habitats, and often impact native species negatively through competition, predation, habitat alteration, disease transmission and hybridization (Ellender *et al.* 2014). It has also been suggested that habitat alteration and other anthropogenic disturbance could influence the impacts that non-native alien introductions will have, and the likelihood of these species establishing (Hermoso *et al*. 2011; Ruesink, 2005).

Predation has often been the cause of native species extinctions where non-native aliens are introduced, especially where the native species may be naive to these 'new' predators, such as was often the case for the lionfish (*Pterois volitans)* in its expansion across the Western Atlantic (Chappell and Smith, 2016). Direct predation can have large impacts on the abundance and distribution of native prey species, but data on these impacts is often scarce (Shelton *et al*. 2015). For example, the non-native rainbow trout *Oncorhynchus mykiss* has been introduced across the Cape Floristic Region of South Africa, yet there is little evidence for the impact these fishes are having (Shelton *et al.* 2015).

However, there are suggestions that they selectively consume small redfin (*Psueudobarbus burchelli)*, depleting their abundance in the areas that they are present (Shelton *et al.* 2015).

Non-native alien species are also known to readily outcompete native individuals, especially where they hold an omnivorous and opportunistic diet. For example, the Nile tilapia (*Oreochromis niloticus*) has been widely introduced across Africa and is invasive in almost every area it has been introduced to, owing to its wide environmental tolerance and broad diet (Canonico *et al.* 2005). Nile tilapia presence has been the cause for large scale species declines and is documented as one of the causes of species decline in Lake Victoria (Dudgeon *et al.* 2006). Outcompeting native species can have knock-on effects for the rest of the ecosystem, for example native species may hold 'specialised' roles and therefore the replacement of these species with 'generalist' alien species can lead to both biotic and functional homogenisation of the habitat (Rahel, 2007; Lepori *et al*. 2012; Marr *et al.* 2010).

The awareness of the impacts these invasive alien species in terms of global biodiversity, but also for the sustainability of recreational and commercial sectors, has increased largely over the past few decades. This awareness has allowed for more attention to be given to investigating possible solutions to this continuing problem. As this issue has been highlighted as one of the least reversible, a lot of research has explored prevention methods (Lind *et al.* 2012b), and local and global legislation is a big part of limiting invasive species dispersal (Piria *et al*. 2018). Blackburn and colleagues (2011) proposed a unified framework that can be applied to all biological invasions and integrates the various branches of literature on the subject. This framework could be a crucial first step to designing appropriate solutions to biological invasions because it recognises that there are a series of stages involved in the process and depending on what stage the particular species or population has reached different management interventions will be needed (Blackburn *et al*. 2011).

One key prevention method has been the establishment of protected areas (MEA, 2016). In a freshwater environment, these protected areas should be independent catchments where non-native species already present (Saunders *et al.* 2002). These protected areas then require whole-catchment management, and maintenance of natural hydrological regimes to prevent admixture with exotic species (Saunders *et al.* 2002). Prevention methods and the allocation of protected areas requires an understanding of species ecology, especially when they are suggested for use in stocking regimes (Alcarez *et al.* 2005; Ruesink, 2005). This understanding will help prevent the further use of species that, while being advantageous for production values, are likely to be highly invasive.

Although the importance of eradicating invasive species is recognised globally, there is still a lack of control mechanisms that completely eradicate the invasive species (Gil *et al.* 2016). For example, mechanical removal of the non-native rainbow trout (*Oncorhynchus mykiss*) from headwater streams across South Africa have not been entirely successful (Shelton *et al*. 2017). Although techniques such as gill netting, angling and fyke netting resulted in significant reductions in population sizes, they did not completely eradicate the species, and populations rose quite rapidly upon cessation of these techniques (Shelton *et al.* 2017). While these techniques need further investigation, it was clear that they may hold some use in reducing the impacts that non-native invasive species can have, and perhaps also in providing employment opportunities in developing countries (Shelton *et al*. 2017), which could have important implications in reducing the conflicts between economic and conservation objectives. Most management strategies involve large costs, such as ongoing monitoring, long-term investments, and the maintenance and development of useful technology.

In Africa, it has been suggested that there is a distinct over-reliance on introduced species such as the Nile tilapia in aquaculture and fisheries management, despite the large diversity of fishes across Africa (Lind *et al.* 2012a; Skelton and Davies, 1986). Recent research on the distribution of these non-native species highlights this over-reliance (Image 1). This species is highly invasive, and yet is some of the most widely used in aquaculture due to their advantageous attributes (Canonico *et al.* 2005). In order to reconcile the conflict between production demands, public opinion, and conservation objectives, aquaculture development must take into account the impact of these non-native alien species (Lind *et al.* 2012a). One management strategy is the design of region-wide zoning of aquaculture practices, whereby farmers are encouraged to farm only indigenous species in their sub-region of Africa (Lind *et al.* 2012a). There has been some evidence to suggest that this might be a successful approach, if used alongside the genetic improvement of native species. For example, artificial selection has led to the improvement of *Oreochromis shiranus* in Malawi (Maluwa and Gjerde, 2007), which is widely cultivated in the country. There is still much to be done in this area, and the use of affordable management techniques could be key to the successful production and sustainability of aquaculture across Africa (Lind *et al.* 2012a).

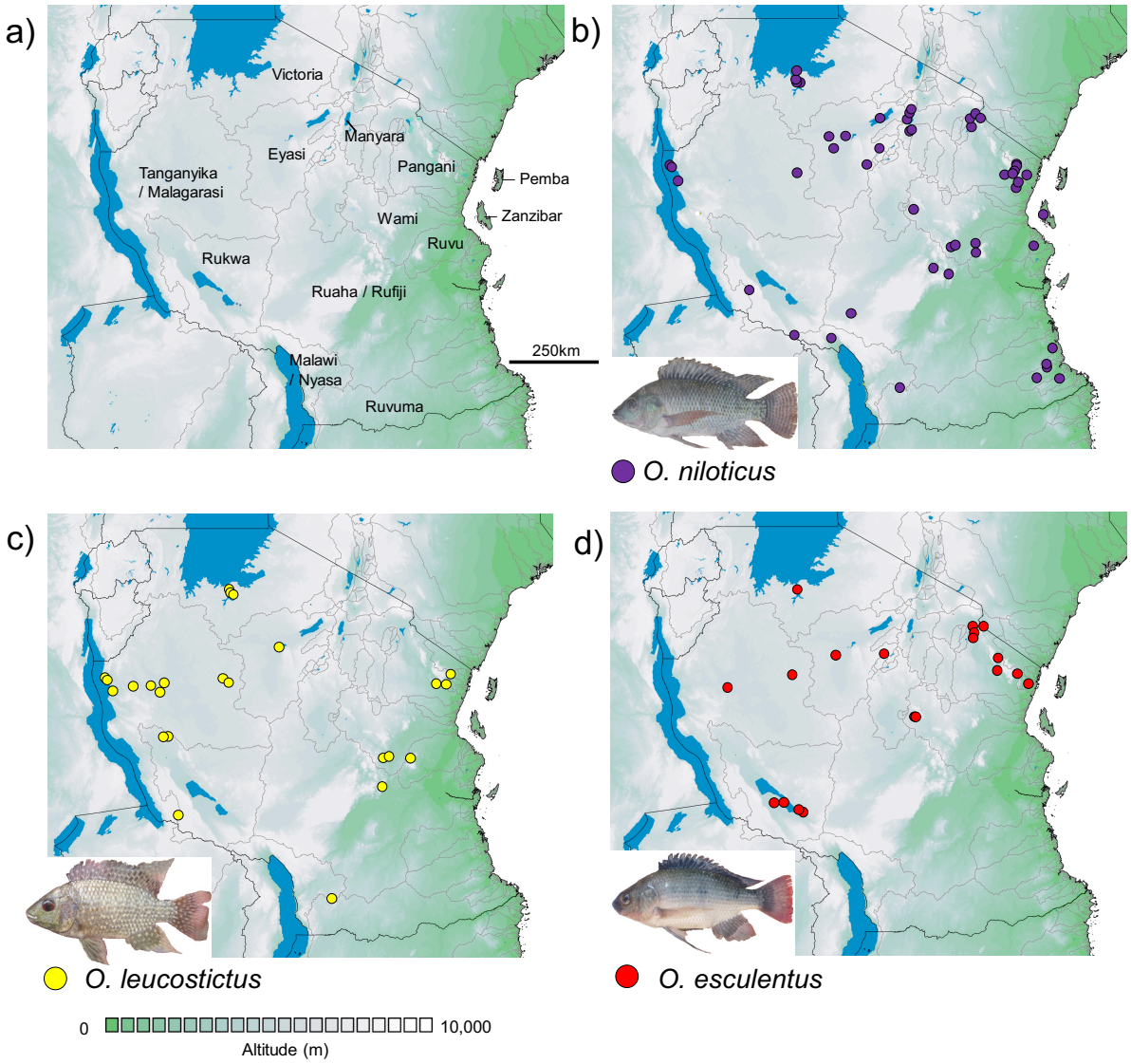

Image. 1 a. Major watersheds of Tanzania, distribution of species introduced beyond their native ranges (*O. niloticus*, *O. esculentus* and *O. leucostictus*; Shechonge *et al.* 2018).

Hybridization

Impacts of hybridization. Hybridization is often referred to as the reproduction between genetically distinct populations (Stebbins, 1959; Todesco *et al.* 2016), and the important role hybridization plays in reducing biodiversity globally has been highlighted (Zengeya *et al.* 2017). Hybridization has led to the extinction of many species both directly and indirectly (Allendorf *et al.* 2001), and there is growing concern for its impacts on biodiversity and the conservation of genetic resources (Rhymer and Simberloff, 1996; Canonico *et al.* 2005). There are two main mechanisms by which hybridization can have negative impacts on genetic diversity; demographic swamping (Wolf *et al.* 2001) and genetic swamping (Todesco *et al.* 2016). Demographic swamping occurs when hybrid progeny have lower fitness than parental individuals (outbreeding depression), which leads to a reduction in population growth rates and eventually can lead to extinction (Todesco *et al.* 2016; Feurtey *et al.* 2017). Genetic swamping occurs when hybrid progeny exhibit increased fitness and instead, over time, replace the pure wild genotypes (Todesco *et al*. 2016). Hybrid progeny are often capable of invading areas that their parental species cannot (Huxel, 1999), and have been shown to perform best in some areas (Levin *et al.* 1996), therefore allowing population sizes to rapidly increase with increased availability for resources.

An interesting example of genetic swamping occurring was reported from Hawaii by Coleman and colleagues (2014), where the introduced Indo-Pacific damselfish (*Abudefduf vaigiensis*) hybridises with the endemic congener *Adudefduf abdominalis*. Hybridization was suggested to be occurring due to exaptation (adaption with unintended consequences) whereby the introduced individuals display a colour pattern year-round that the native species only display during reproduction as a signal of receptivity (Coleman *et al.* 2014). Hybrid individuals often display the same year-round colouration of the introduced species (Coleman *et al*. 2014). The large number of later generation hybrids found suggested that genetic swamping of the native species may be occurring and presents a critical threat to the persistence of the endemic *A. abdominalis.*

There are many models aimed at predicting the consequences of hybridization and introgression to assess the need for management. However, they often assume that mating is random (e.g. Huxel, 1999), which is often not the case. For example, in many freshwater fishes, females show clear assortative mating that in some cases acts as a reproductive barrier preventing hybridization (Schumer *et al.* 2017). On the other hand, in some cases, mate choice behaviour can instead increase the prevalence of hybridization events, for example in the case of the allopolyploid fish *Squalius*

alburnoides, where female mate preferences for heterospecifics leads to the persistence of hybrids within the population (Morgado-Santos *et al.* 2015). Clearly, mate choice plays a key role in the outcome of hybridization, as while assortative mating may reinforce reproductive isolation, it can often break down, therefore allowing the persistence and success of hybrids (Schumer *et al.* 2017).

A recent review suggested that genetic swamping is more often the concern from hybridization events, than that of demographic swamping (Todesco *et al.* 2016). However, it is often difficult to disentangle the two, and in many cases both mechanisms may be occurring (Wolf *et al.* 2001). For example, in small isolated populations, when the hybrid progeny are viable but infertile, hybridization is much more likely to have detrimental effects, and high levels of hybridization are likely to lead to genetic and demographic swamping (Roberts *et al.* 2009). In Southeast Australia, hybridization occurs between the obligately estuarine black bream (*Acanthopagrus butcheri*) and the migratory marine yellowfin bream (*Acanthopagrus australis*; Roberts *et al.* 2009). Research on this phenomenon has shown that hybrid numbers have remained stable over several years, and hybrid individuals persist through to adulthood and are long-term residents within lagoons, while pure *A. butcheri* are virtually absent (Roberts *et al.* 2009; Roberts *et al.* 2010). In these instances, the high levels of introgression in several hybrid zones suggest that demographic and genetic swamping poses a major threat to the persistence of these populations (Roberts *et al.* 2010).

Hybridization has been suggested to be one of the most rapidly acting genetic threat to populations, as it can lead to the extinction of species within four or five generations (Huxel, 1999). This in itself is enough to cause concern for many systems, but predictions for the future suggest that rates of hybridization will continue to increase (Todesco *et al.* 2016), with increasing land use for agriculture (Kalacska *et al*. 2017), the rising introduction of non-native species for uses such as aquaculture and fisheries stocking programs (Lind *et al.* 2012a; Lind *et al.* 2012b) and the growing concern of climate change affecting species ranges (Bush *et al.* 2014).

Anthropogenic land use is a major factor contributing to increased occurrence of hybridization in freshwaters (Mantel *et al*. 2017; Coleman *et al*. 2014). For example, investigation of hybridization and introgression between blueback herring (*Alosa aestivalis*) and alewife (*Alosa pseudoharengus*) suggested markedly different patterns in landlocked populations compared to anadromous populations, with all landlocked individuals being hybrids (Hasselman *et al*. 2014). These findings suggested that anthropogenic land use changes, such as the creation of dams, can allow reproductive barriers to break down, creating hybrid swarms (Hasselman *et al.* 2014). With the predicted increase

in dam construction globally (Kalacska *et al.* 2017), such hybrid swarms could become an increasing problem. The removal of unnecessary or old dams could be an appropriate solution here (Mantel *et al*. 2017).

In the review by Todesco and colleagues (2016), they established that 39% of research suggesting a threat of extinction via hybridization included non-native invasive taxa. The likelihood of hybridization events leading to native extinction depends on a number of factors, for example pre-zygotic and post-zygotic reproductive barriers (Wolf *et al*. 2001). As invasive species are introduced beyond their range, they will not have co-evolved with native species, therefore reproductive and isolating mechanisms are less likely to be complete (Todesco *et al.* 2016). This means that native species are not only threatened by competition with non-natives, but also by the consequences of continued hybridization occurring (Wolf *et al.* 2001; Huxel, 1999).

Research by Deines and colleagues (2014) found an interesting situation whereby the native cichlids *Oreochromis macrochir* and *Oreochromis andersonii* were hybridising in the presence of the invasive *O. niloticus*. The two native species do not naturally hybridise when in sympatry, which suggests that the presence of the non-native species is facilitating these hybridization events (Deines *et al.* 2014). Most interbreeding seems to be occurring between fish of mixed ancestry, and between those mixed individuals and the parental species *O. macrochir* and *O. andersonii,* creating a complex hybrid swarm with few pure parental genotypes remaining in the population (Deines *et al.* 2014; Harrison, 1990). This research highlights the impact non-native tilapia introductions may be having on the genetic resources available for aquaculture, and the impact of hybridization is often not considered in management decisions.

Intraspecific hybridization is another cause for concern, for example where cultured fish from aquaculture farms escape and mix with wild populations (D'Amato *et al*. 2007). Where it is thought that this type of hybridization should only cause a minor shift in genotype frequencies due to populations generally sharing alleles (Allendorf *et al.* 2001), this argument ignores population genetic complexity. In cases where population genetic structure is prevalent, the frequency of particular phenotypes may become more and more different between populations, as the number of loci involved increases (Allendorf *et al.* 2001). Specifically, in cases where intraspecific hybridization is due to escapes from cultured populations, it is likely that the cultured individuals may have altered genotypes due to artificial selection and domestication (Ambali *et al.* 1999; Grant, 2012). For example, *Oreochromis shiranus* saw a considerable reduction in the number of alleles per locus even

in the early stages of domestication for aquaculture (Ambali *et al.* 1999). Cultured species are often specifically adapted to the culture environment, and in the wild these characters could become maladaptive, therefore hybridization events with wild congeners could introduce deleterious alleles into the population (Brummett and Ponzoni, 2009; Grant, 2012).

Hybridization events do not always lead to extinction, and can sometimes increase genetic diversity, for example by introducing new alleles into small populations (Todesco *et al.* 2016). It has been suggested that the maintenance of hybrid zones may not be due to reduced hybrid vigour, but due to their adaptation to environments different to those occupied by parental species (Barton, 2001). Seehausen (2004) noted that many cases of introgressive hybridization between species can be found in rapid adaptive radiations, such as *Heliconius* butterflies (Gilbert, 2003; Seehausen, 2004), Darwin's finches (Grant *et al*. 2003), and the cichlid fishes of the African Great Lakes (Buress, 2015). Natural selection on the genome plays a crucial role in the extent to which hybridization can promote speciation (Albertson and Kocher, 2005). Models have predicted that introgressive hybridization is most likely to lead to adaptive radiation where vacant ecological niches are highly dissimilar to the parental niches, and there is moderate genetic differentiation between parental species (Kagawa and Takimoto, 2018).

Hybrid speciation is gaining more attention, and there is evidence to suggest that ancient hybridization events have led to the speciation of many new species, and that ongoing hybridization events are not as uncommon as previously thought (Koblmuller *et al.* 2007; Seehausen, 2004). Several cichlid groups, for example the lamprologine cichlids of Lake Tanganyika, have been found to have experienced recent hybridization events without any deterioration of phenotypic diversity (Koblmuller *et al.* 2007). Authors have also highlighted the role of introgression in Darwin's finches (Arnold and Hodges, 1995), and here the environmental shifts caused by El Niño and La Niña have allowed hybrid genotypes to exhibit increased fitness compared to parental types, in some cases reducing reproductive isolation between species (Grant and Grant, 2002). These findings highlighted that reticulate evolution may explain several lineages within this classic model system (Arnold and Martin, 2010), and suggest that hybridization may be an important aspect of the natural evolution of many species.

While adaptive radiation and the production of genetic and phenotypic novelty resulting from some hybridization events may have positive evolutionary consequences, these factors have also been linked to the evolution of invasiveness in hybrids (Ellstrand and Schierenbeck, 2006). Several studies

have investigated the link between invasion success and hybridization, whereby evolutionary novelty, genetic variation and heterosis may have increased the likelihood of invasion success (Ellstrand and Schierenbeck, 2006). In freshwater fishes, hybridization is regularly documented and has often seen increased growth rates, as well as other factors that may increase their success, including disease resistance and environmental tolerances (Bartley *et al.* 2001; Wilcox *et al.* 2018). This may even be the case for marine fishes, as Wilcox et al. (2018) discovered that invasive *Pterosis* lionfish in the Atlantic are likely to be of hybrid origin, and its dramatic spread may be due to hybrid vigour (heterosis) allowing increased invasion success.

Occurrence of hybridization. Hybridization was once thought to be relatively rare in natural systems, occurring mainly in plant species. However, evidence showing natural hybridization events occurring in many animal taxa suggests it occurs much more commonly than previously thought (Mallet, 2005). Hybridization occurs most commonly in freshwater fish, compared to all other vertebrate species (Scribner *et al.* 2000; Allendorf *et al.* 2001). Taxonomic bias in the extent of hybridization is well known in both marine (Montanari *et al.* 2016) and freshwater fishes (Scribner, 2000). This bias is likely attributable to higher relatedness of species, with those families that have a high proportion of recently diverged species more likely to hybridise (Scribner, 2000). For example, among the freshwater fishes, hybridization occurs most commonly in the cyprinids, because they exhibit high rates of adaptive radiation and species are often very closely related (Parveen *et al.* 2018). Cyprinid taxa commonly produce fertile offspring (Rognon and Guyomard, 2003), and are able to backcross with parental species, leading to introgression (Parveen *et al*. 2018).

Hybridization events occur widely due to anthropogenic disturbance, and the introduction of non-native species through human activities such as aquaculture (Scribner, 2000). Hybridization is a common occurrence when non-native species are introduced to stock water bodies (Lind *et al.* 2012b). Tilapias are a widely used taxa in aquaculture (Conte *et al*. 2017), particularly across Africa, and their propensity to hybridise has increased the occurrence of hybrid zones (Rognon and Guyomard, 2003). While aquaculture in Africa is relatively young (Brummett and Williams, 2000), international and local markets for farmed tilapias are growing (Brummett and Ponzoni, 2009). The growth in this sector has led to an increase in species introductions, which often lead to hybridization events (Brummett and Ponzoni, 2009). In Africa, *O.niloticus* is one of the most popular species for aquaculture (Eknath *et al.* 2009), and many incidences of either accidental or intentional hybridization have been reported (Brummett and Ponzoni, 2009). For example, *O. niloticus* mtDNA has been found in several other species across Africa, including *O. aureus* (Agnese *et al.* 1997; Rognon and

Guyomard, 2003), *O. esculentus* (Angienda *et al*. 2011), *O. andersonii* and *O. macrochir* (Deines *et al.* 2014), and *O. leucostictus* (Nyingi and Agnese, 2007). In one example of *O. niloticus* and *O. leucostictus* hybridising, the two species were apparently not sympatric, and it is still unknown how *O. leucostictus* may have been introduced to the *O. niloticus* habitat (Nyingi and Agnese, 2007). This example highlights that more stringent monitoring is needed to control the movement of these readily-hybridising species.. In some cases, tilapia populations contain genes of multiple other species (McAndrew *et al.* 1988; Brummett and Ponzoni, 2009). These outbreeding events can sometimes lead to a reduction in preferred traits, such as growth rates or sex determination (Brummett and Ponzoni, 2009).

Research suggests that the negative impacts of aquaculture practices most often occur where there has been little consideration of the genetic consequences of domestication, where stocks are transferred among farms unscrupulously, and without any kind of selective breeding (Ambali *et al.* 1999). Ambali et al. (1999) suggested that the socio-economic status of farmers not only caused cultured populations of *Oreochromis shiranus* to show reduced genetic diversity, but also allowed introgression to occur between *O. mossambicus* and the cultured *O. shiranus* (Ambali *et al*. 1999). The vast majority of aquaculture occurs in developing countries where there is little knowledge of the genetic characteristics of cultured species, and unmonitored inter-basin exchange occurs widely (Barasa *et al.* 2014). This might suggest that hybridization events are much more widespread than initially thought.

Genetic improvements are a large part of increasing yield in aquaculture practices, and one of the most common practices in areas with limited resources, for example in Thailand one of the most common practices to improve genetic performance is the use of hybridization (Na-Nakorn and Brummett 2009). The African sharptooth catfish *Clarias gariepinus* is one of the largest aquaculture species in Asia and has been introduced for use in aquaculture to at least 35 countries (Froese and Pauly, 2018). The species is used as a pure species, but is also used to produce hybrids with other catfish species such as *Heterobranchus longifilis* (Na-Nakorn and Brummett 2009). While other selective breeding programmes have been relatively unsuccessful, the mix of *C. gariepirus* and *H. longifilis*, known as '*heteroclarius*' has successfully taken advantage of the culinary quality of the former species and the high growth rates of the latter species (Na-Nakorn and Brummett, 2009). Little is known about the impacts of these hybridization events on the native fish communities in Thailand, but the establishment of these species and their hybrids has been thought to impact the endemic cyprinids of Lake Lanao in the Phillipines (Na-Nakorn and Brummett 2009). Other common

hybridization of clariid species includes that of *Clarias macrocephalus* and *C. gariepinus*, which has been used for more than 20 years across Asia (Koolboon *et al.* 2014). Recently, the first report of the successful hybridization of the African catfish (*C. gariepinus*) and the Asian catfish (*Pangasianodon hypophthalmus*) was published (Okomoda *et al.* 2017). This research highlighted phenotypic variation in hybrid progeny, that will likely become the backbone of future investigations into the use of these hybrids for aquaculture (Okomoda *et al*. 2017).

Another example of the use of hybridization to improve cultured strains is that of the Mozambique tilapia (*Oreochromis mossambicus*; D'Amato *et al*. 2007), which is widely used in aquaculture across Africa (Taylor *et al.* 2017). *Oreochromis mossambicus* was one of the first tilapia species to be widely used for aquaculture (Taylor *et al.* 2017), and due to its broad salinity tolerance it is often hybridised with other *Oreochromis* species for use in aquaculture (Moralee *et al.* 2000). Many examples of hybridization events between *O. mossambicus* and other tilapia species have been reported in the natural environment (Lind *et al*. 2012a). For example, D'Amato and colleagues (2007) found genetic evidence of *O. mossambicus* hybridization with *O. niloticus* in the Limpopo river system, and *O. mossambicus* is now recognised by the IUCN as an endangered taxon due to its hybridization with the invasive *O. niloticus* (Firmat *et al.* 2013). However, there is still some promise for the conservation of the Mozambique tilapia, as populations have been discovered with no traces of introgression with non-native species (Firmat *et al.* 2013). These findings suggest that these refugial zones could represent valuable conservation value and provide genetic resources for the continued aquaculture in the area (Firmat *et al*. 2013). Notably, *O. mossambicus* is also hybridised with *O. niloticus* in aquaculture to produce red tilapia, which is preferred in some markets due to its vibrant red colouration and improved taste (Moralee *et al.* 2000).

While hybridization events occur frequently among tilapiines within aquaculture breeding programmes and following species introductions of non-native species into the natural environment, verified incidences of hybridization between sympatric indigenous species are much less common. Among the few documented cases of hybridization among native species is between *Coptodon zillii*, *Coptodon guineensis* and *Coptodon dageti* in West Africa (Pouyaud and Agnese, 1995; Rognon and Guyomard, 2003), and between *Orechromis jipe* and *Oreochromis korogwe* in Tanzania (Bradbeer *et al.* 2018).

Methods used to detect hybrid individuals: Meristic and morphological measures have traditionally been used to identify hybrids, as it is assumed that hybrid progeny will portray intermediate morphologies to the parent species (Trewavas, 1983). For example, Trewavas (1983) analysed the morphology of hybrid crosses between the aquarium stock of *Sarotherodon melanotheron* and male *Oreochromis niloticus*. Morphological techniques for identifying hybrids can be successful (Allendorf *et al.* 2001). For example, the initial identification of hybrids between cyprinid species in the Durance river, a system highly segregated with multiple dams, was achieved using a combination of both morphological and allozyme analysis (Costedoat *et al.* 2005). Coloration has also been used successfully as an indicator of putative hybrids, for example hybrids of the tilapias *C. zillii* and *C. guineensis* have three diagnostic phenotypic characters, namely a yellowish caudal fin, a bicoloured caudal fin, and a bicoloured caudal fin with spots (Nobah *et al.* 2008).

The use of morphological techniques often assumes that hybrid progeny will exhibit intermediate phenotypes between the two parental species, and this was once an almost universal rule when identifying hybrids (Lamb and Avise, 1987; Hubbs, 1955). The establishment of a morphological 'hybrid index' has allowed researchers to make estimations of different hybrid classes, as the individuals showing 'intermediate phenotypes' would be categorised as F1 hybrids, and any other deviations as F2 or backcrosses (Lamb and Avise, 1987; Hardig *et al.* 2000). However, morphological hybrid indexes do not always allow accurate identification (Lamb and Avise, 1987), as phenotypic characters can overlap between closely related species, especially where morphological differences are slight (Walsh *et al*. 2015). Moreover, the assumption of an 'intermediate phenotype' is also not always valid (Walsh *et al.* 2015), as selection pressures cannot always be predicted, and levels of introgression may vary among loci (Walsh *et al.* 2015). In some instances, hybrid progeny exhibit transgressive segregation, whereby they show extreme or novel phenotypes (Seehausen, 2004), which can be much easier to identify (Burress, 2016). In other cases, phenotypes can be spread across a gradient that overlaps substantially with parental phenotypes (Gay *et al.* 2008; Walsh *et al.* 2015).

While first generation hybrids are often intermediate in many morphological measures between both parental species, selection against the recombination of these phenotypes can lead to a general underestimation of hybrid progeny in a population (Rieseberg *et al.* 1999a; Rieseberg *et al.* 1999b; Wolf *et al*. 2001), and morphological measures typically give no indication of whether the individual is a first-generation hybrid, a backcross, or even a later generation hybrid (Allendorf *et al*. 2001). Various population genetic work suggests that morphological measures can often be misleading and

should only be used as a preliminary step in identifying hybrids (e.g. D'Amato *et al*. 2007; Bradbeer *et al.* 2018).

Where the use of morphological measures has not always provided a clear picture in detecting hybrids, the use of genetic methods alongside these practices has allowed a much more successful and accurate assessment in most cases (Sales *et al.* 2018; Parveen *et al.* 2018, etc). In the mid 1960's the development of allozymes allowed genetic determination of hybrid individuals, making the process much simpler (Allendorf *et al*. 2001). Advances in molecular techniques such as the use of microsatellites, mitochondrial DNA and whole genome sequencing/genotyping have allowed much more success in the identification of hybrids and the interpretation of the role they play in the evolution or extinction of a variety of species (O'Connell and Wright, 1997). The development of genetic markers in hybridization research also allows for analysis of the underlying causes of hybridization, as well as the extent and direction of these events (Scribner, 2000).

Microsatellites, or "Simple Sequence Repeats" (O'Connell and Wright, 1997) are often highly polymorphic (Evanno *et al.* 2005; Jarne and Lagoda, 1996), and provide a powerful tool for identifying hybrids in the first few generations (Ambali *et al.* 1999). However, there are still some concerns, for example, the accurate detection of hybrids has been found to be dependent on the number of microsatellite markers used (Henriques *et al.* 2016), and they can still be fairly expensive to develop and difficult to use (Evanno *et al*. 2005). Mitochondrial DNA can also be very useful in the identification of hybrids, as well as detecting the direction of hybridization as mtDNA is maternally inherited (Rhymer and Simberloff, 1996). Mitochondrial DNA has been used in various studies alongside the use of nuclear DNA markers to successfully identify hybrid progeny from parental species (e.g. Rognon and Guyomard, 2003).

To make inferences about the outcomes, timescale and magnitude of hybridization using these genetic markers, various statistical methods have been developed and incorporated into widely used software packages. These packages include NEWHYBRIDS (Deines *et al.* 2014), STRUCTURE (Hubisz *et al.* 2009; Deines *et al.* 2014), and the R package HYBRIDDETECTIVE (Wringe *et al*. 2017). These software packages are particularly useful because they do not necessarily require diagnostic alleles to be identified, and do not always require pure parental individuals to be available for the analysis (Parveen *et al.* 2018).

As well as detecting hybrids, it is also important to determine the causes of these events, in order to apply appropriate management schemes (Richards and Hobbs, 2015). For example, habitat degradation and other anthropogenic threats often promote hybridization (Dudgeon *et al.* 2006; Lind *et al.* 2012a) and if this is the case thorough management will be needed. Identifying genetically important units of conservation is a new method used to interpret the need for conservation in the face of threats such as hybridization (Hallerman and Hilsdorf, 2014), as in some instances hybridization events have proven beneficial promoting adaptive evolution (Albertson and Kocher, 2005), and in others leads to the extinction of endemic species (Coleman *et al.* 2014).

The Project:

Cichlid fishes are the most species rich family of freshwater fishes on the planet (Buress, 2015). The cichlid flocks of the Great Lakes of East Africa present one of the best examples of rapid speciation and adaptive radiation (Salzburger *et al.* 2014). African cichlid diversity is dominated by two major groups; the haplochromines and the tilapiines (Trewavas, 1983). Tilapiine fishes are a group comprising eleven genera; *Alcolapia*, *Danakilia*, *Heterotilapia*, *Iranocichla*, *Oreochromis*, *Pelmatolapia, Sarotherodon, Steatocranus*, *Stomatepia*, *Tilapia*, and *Tristramella* (Trewavas, 1983). Molecular phylogenetic evidence suggests these are a paraphyletic group within a broader African cichlid clade (Dunz and Schliewen, 2013), but the common term 'Tilapia' is still widely used to refer to the species across these genus (Trewavas, 1983). Collectively, the 'Tilapia' are the most important fishes for aquaculture and inland fisheries in Africa (Liu *et al.* 2013). Of the tilapiine genera, the mouth-brooding genus *Oreochromis* is the most species rich, with 32 valid species found across a range of freshwater and estuarine habitats across the continent (Froese and Pauly, 2018).

While there has been a large amount of research on the cichlid flocks of the African Great Lakes, relatively little work has been done on smaller lakes and river systems across Africa (Firmat, 2013), particularly the tilapia fishes. This project focusses on the morphological and genetic characteristics of *Oreochromis korogwe* (Lowe, 1955), a relatively small-bodied species endemic to Tanzania. Until relatively recently, *Oreochromis korogwe* was known only from coastal river systems of northern Tanzania, namely the Pangani and Zigi river systems (Trewavas, 1983), and Mlingano dam near Tanga (Trewavas, 1983; Lowe-McConnell, 2006). Suggestions that the species occurs in Lake Chala, on the border of Tanzania and Kenya are likely erroneous. The large bodied taxon found there is plausibly an introduced population of *O. urolepis* (see Dieleman *et al.* 2018). Recent research investigating the distribution of *Oreochromis* species discovered individuals of the *O. korogwe* in

three lakes in southern Tanzania, namely Lakes Rutamba and Nambawala in 2013, and Lake Mitupa in 2016 (Image 2; Shechonge *et al.* 2018). The cichlid fishes from this area have been poorly studied, and it is notable that museum specimens of the species from Lake Rutamba, the largest of the three lakes, were historically inaccurately assigned to *Oreochromis placidus* (Trewavas, 1983).

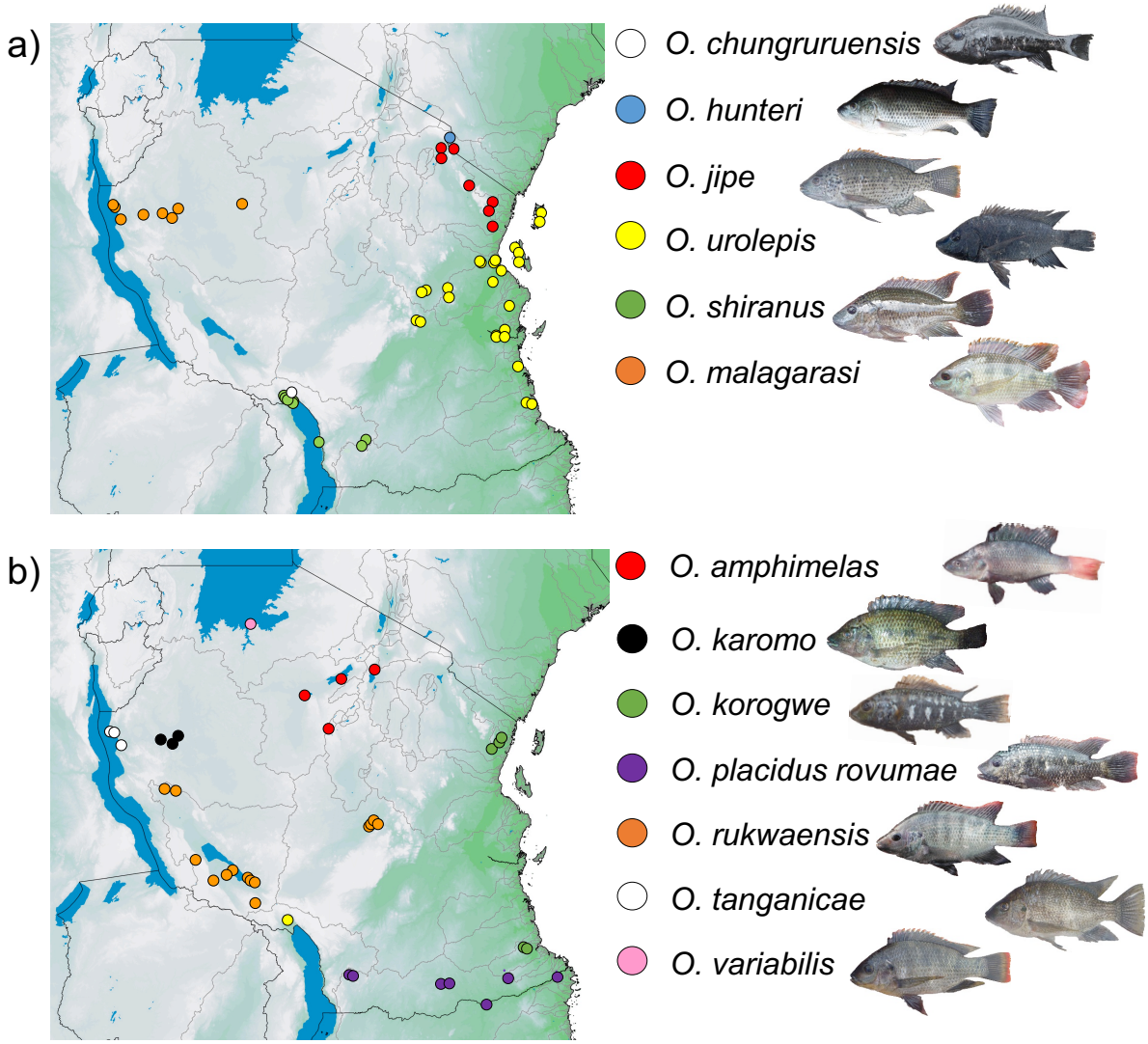

Image. 2 Distribution of native Oreochromis species across Tanzania (Shechonge *et al.* 2018).

The presence of these populations so far south of their previously reported distribution (Trewavas, 1983) highlights the need to compare these populations with northeastern populations, as it is plausible these are populations that diverged sufficiently to be considered as distinct species. This project aims to compare *O. korogwe* populations using microsatellite-based population genetic analyses, traditional morphological measurements, and geometric morphometric analysis, in order to test for distinguishing characters of these populations and determine if they are indeed conspecific or instead distinct species.

Alongside *O. korogwe* individuals in the southern lakes is the invasive alien species *O. niloticus* (Shechonge *et al.* 2018). This species is one of the most popular species for aquaculture in Africa (Conte *et al.* 2017) and has one of the broadest natural distributions among freshwater fishes (Bezault, 2011), due to extensive introductions beyond its native range (Agnèse *et al.* 1997). Recent research has found individuals of *O. niloticus* in all major river basins of Tanzania, largely due to deliberate stocking for the improved production of fisheries in the region (Shechonge *et al*. 2018). While *O. niloticus* is native to Lake Tanganyika in Tanzania, they were first introduced to Lake Victoria in the mid 1950s from elsewhere in the Nile system (Ogutu-Ohwayo, 1990), and subsequent stocking practices often use the non-native population from Lake Victoria rather than the native Lake Tanganyika population (Shechonge *et al.* 2018).

Nile tilapia has a broad, opportunistic diet, and is tolerant of a wide range of water temperatures and salinity, making them highly invasive in these new areas (Canonico *et al.* 2005). The species is well known to hybridise with wild congeners (Moralee *et al.* 2000), often producing viable and fertile progenies (Rognon and Guyomard, 2003), and contributing to hybrid swarms contributing to the loss of unique genetic diversity in native species (Deines *et al.* 2014). *Oreochromis niloticus* is likely to have assisted the widespread extirpation of native *Oreochromis* species from the main body of Lake Victoria, namely *O. esculentus* and *O. variabilis* (Ogutu-Ohwayo, 1990). The presence of *O. niloticus* in the southern lakes Rutamba, Nambawala, and Mitupa presents a threat of the survival of these newly discovered populations of *O. korogwe* both from competition (e.g. Oso *et al*. 2006), and hybridization (e.g. Conte *et al.* 2017). The current work aims to investigate whether *Oreochromis niloticus* is hybridising with the newly discovered *O. korogwe* populations, and whether any resultant hybrids can be distinguished from purebreds using either traditional or geometric morphological analysis. If hybridization is occurring between the two species, it is likely that the potentially unique genetic resources of the newly discovered *O. korogwe* populations are threatened. This work will present a crucial first step in determining the need for conservation in this area.

Chapter 2: Paper Chapter

Newly discovered tilapia cichlid fish biodiversity threatened by hybridization with non-native species.

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My contribution to this data chapter included collection of all southern population samples, all laboratory work on southern samples, and of DNA extracts of northern specimens collected in 2013, and 2015. All statistical analysis was conducted by me, and all ideas and opinions expressed are my own.
Signed:
Tabitha Richmond

27 July 2018

Abstract

Non-native species introduced into freshwater systems can readily hybridize with indigenous congeneric species. In tilapiine cichlids this hybridization can lead to loss of both unique biodiversity and irreplaceable genetic resources. Here we tested if newly discovered (2013-2016) populations of *Oreochromis korogwe* from southern Tanzania are undergoing hybridization with the larger invasive Nile tilapia (*Oreochromis niloticus*). We sampled three lakes containing *O. korogwe* and *O. niloticus*, and quantified genetic identity using microsatellite loci. We found evidence for the presence of hybrids in the three lakes (Nambawala 27% hybrid; Lake Rutamba 6% hybrid; Mitupa 29% hybrid). We also found that while genetically purebred forms could be discriminated using head and body shape information, these external morphometric measurements alone cannot reliably be used to identify hybrids. We next explored genetic and morphological differences between the geographically disjunct populations of *O. korogwe* from northern Tanzania (Zigi River and Mlingano Dam) and purebred *O. korogwe* in the southern lakes. Our results show clear genetic differences between the northern and southern populations, that also differed in multiple aspects of body and head shape. We conclude that newly-discovered phenotypically unique populations of a tilapiine cichlid are threatened by hybridization with an invasive species and propose that future introductions of non-native species are prevented in African water bodies that contain irreplaceable genetic resources.

Key words: Introgression, biodiversity conservation, cichlid fishes, population genetics, geometric morphometrics.

Introduction

Hybridization is a widespread phenomenon that has been well studied in many groups of organisms, including plants, insects and vertebrates (Mallet, 2005). Often hybridization leads to the sharing of allelic diversity among species that maintain assortative mating, and in such cases hybrid individuals typically possess relatively low fitness compared to the parental species. However, hybridization can lead to new allelic combinations that promote adaptive resilience to environmental change (Malinsky et al. 2015). For example, research into Darwin's finches has shown that hybrid progeny can have higher fitness than parental species following periods of environmental shifts (Grant *et al.* 2003). Despite the growing evidence for the role that hybridization has played in evolution, hybridization is most often considered to be a threat to biodiversity, leading to loss of genetically unique natural populations through genetic or demographic swamping (Roberts *et al.* 2010; Todesco *et al.* 2016). In fish, in particular, hybridization has been invoked as a driver of local extinction, often exacerbated by human activities such as habitat degradation and species introductions (Allendorf *et al.* 2001).

Freshwater ecosystems are among the most diverse on earth, however they are also the most threatened (MEA, 2016). They are undergoing rapid changes in biodiversity due to the interacting effects of habitat degradation, over-exploitation, water pollution, species invasion, water abstraction and flow modification (Sala *et al.* 2000; Dudgeon *et al.* 2006). As human population sizes continue to rise, and climate change becomes an ever-increasing threat, these impacts are predicted to grow (Martinuzzi *et al.* 2014; Arroita *et al*. 2017; Kalacska *et al.* 2017). Relative to these threats, the potential for hybridization between introduced non-native alien species and native species has been relatively poorly studied but could become an increasingly important issue with the spread of freshwater species for aquaculture and inland fisheries enhancement (Deines *et al.* 2014).

In Africa, inland capture fisheries across the continent are now maximally or overexploited, and thus aquaculture is predicted to expand dramatically over the coming decades to meet the protein needs of the growing human population. One of the most valuable resources for both fisheries and aquaculture are the "Tilapias" (Brummett and Williams, 2000), a group of cichlids that includes the commercially-important genera *Oreochromis, Sarotherodon, Tilapia and Coptodon*. Among the most prominent species used across Africa in aquaculture and capture fisheries improvement programmes is the Nile tilapia (*Oreochromis niloticus*), which accounts for almost 75% of global tilapia production (Deines *et al.* 2014). Notably, the species has readily colonized new non-native habitats and become invasive

in at least 114 of the 140 countries where it has been introduced (Deines *et al.* 2016). The invasive success of this species has been ascribed to fast growth, broad physiological tolerances of a variety of environmental conditions, and an omnivorous diet (Canonico *et al.* 2005; Ogutu-Ohwayo, 1990). In Africa, Nile tilapia is also known to hybridize with native *Oreochromis* species at the locations where it has been introduced, for example with *O. mossambicus* in Southern Africa (D'Amato, 2007), and *O. esculentus* in Lake Victoria (Angienda *et al.* 2011). However, despite the growing concern surrounding the impacts hybridization on native *Oreochromis* populations, the potential loss of unique native genetic resources due to hybridization with Nile tilapia remains poorly studied.

The availability of genetic resources from wild relations of farmed species is likely to take a crucial role in the long-term viability of aquaculture and inland fisheries production (Brummett and Ponzoni 2009). The availability of wild relatives of farmed species can allow for the incorporation of favorable traits into broodstock, such as high growth rates, large body sizes, efficient food conversion ratios and disease resistance (Bentsen *et al.* 2017; Ndiwa *et al.* 2014; Bernatchez *et al.* 2017). This is particularly important where the culturing and domestication process leads to diminished genetic variation in production systems (Brummett and Ponzoni, 2009). Thus, the preservation of wild genetic resources may be important as tilapia aquaculture expands. However, many tilapia species are range restricted and considered imperiled (e.g. Shechonge *et al.* 2018), and the loss of the unique genetic diversity held by these species could lead to cascading impacts on food security, locally and globally (FAO, 2016). To conserve these resources for the future growth and stability of fisheries and aquaculture, as well retaining the intrinsic value of biodiversity, management systems need to take these growing concerns into account (Hallerman and Hilsdorf, 2014).

Recently (2013 and 2016), populations of *Oreochromis korogwe* were discovered in three lakes in southern Tanzania near Lindi (Lakes Rutamba, Nambawala and Mitupa; hereafter referred to as 'southern populations'). Previously this species was only known from the Pangani and Zigi river catchments in northern Tanzania (hereafter referred to as 'northern populations'), some 500 km north of Lindi (Shechonge *et al.* 2018, Trewavas, 1983). The rivers between Lindi and the Pangani are populated naturally only by *O. urolepis*. Such a large geographic discontinuity in the apparent natural distribution of *Oreochromis* is not known in any other species (Trewavas 1983, Shechonge *et al.* 2018), and is rare in other African freshwater fishes (e.g. Skelton and Davies, 1986). Importantly, in all three of the southern lakes studied, the non-native species *Oreochromis niloticus* was also found. This study aims to: 1) Investigate the extent to which these newly discovered southern populations of *O. korogwe* have hybridized with the non-native individuals, and 2) quantify the genetic and

morphological differences between the northern and southern populations of *O. korogwe*. Both geometric morphometrics and traditional linear morphometric measurements are used to quantify differences between these populations, together providing a clearer understanding of often subtle differences in morphology (e.g. Shukla and Bhat, 2017). The results are discussed with reference to the conservation of newly discovered unique endemic genetic resources, and to the drivers of evolutionary divergence among populations.

Materials and Methods

Study sites and sample collection.

Oreochromis korogwe, *O. niloticus* and their potential hybrids were collected from the southern Lake Rutamba, Lake Nambawala, and Lake Mitupa between 23-27 October 2016 (Figs. 1-2; Table 1-2). For genetic analyses specimens were also used from collections in 2013 (Genner, M.J.) and 2015 (Shechonge *et al.* 2018). Samples of northern *O. korogwe* were collected from the Zigi River and Mlingano Dam on the 18 August 2015 (Figs. 1-2; Table 2). Samples were collected either using multi-mesh gill nets, a seine net, or from purchasing from local fishermen. Multimesh nets measured 30m in length with a stretched depth of 1.5 m height, and 12 panels each 2.5 meters long. Mesh sizes for panels were in the following order 43 mm, 19.5 mm, 6.25 mm, 10 mm, 55 mm, 8 mm, 12.5 mm, 24 mm, 15.5 mm, 5 mm, 35 mm and 29 mm. The seine net measured 30 m in length, 1.5 m in height with 25.4 mm mesh and fine mesh cod end.

Table 1. Sample sizes for southern comparison analysis of *O. korogwe*, *O. niloticus*, and individuals of hybrid origin*.

Site	Species	Genetic Analysis	Linear (traditional) measures	Geometric morphometric
Lake Mitupa	*O. korogwe (OK)*	2	-	-
	O. niloticus (ON)	3	3	3
	Hybrid (OK x ON)	2	1	1
Lake Rutamba	*O. korogwe*	17	9	9
	O. niloticus	13	6	6
	Hybrid (OK x ON)	2	2	2
Nambawala	*O. korogwe*	10	9	9
	O. niloticus	6	5	5
	Hybrid (OK x ON)	6	5	5

*Note that while genetic analyses were based on a range of samples collected in 2013, 2015 and 2016, morphological analyses were only conducted on samples collected in 2015, and 2016.

Table 2. Sample sizes for comparisons of southern and northern *O. korogwe* populations and reference populations of *O. urolepis* and *O. placidus*.

Site	Species	Genetic Analysis	Linear (traditional) measures	Geometric morphometric
Mlingano dam	*O. korogwe*	40	34	40
Zigi River	*O. korogwe*	16	23	29
Lake Chidya	*O. placidus*	10	-	-
Rufiji River	*O. urolepis*	26	-	-
Lake Nambawala	*O. korogwe*	10	9	10
Lake Rutamba	*O. korogwe*	17	14	9

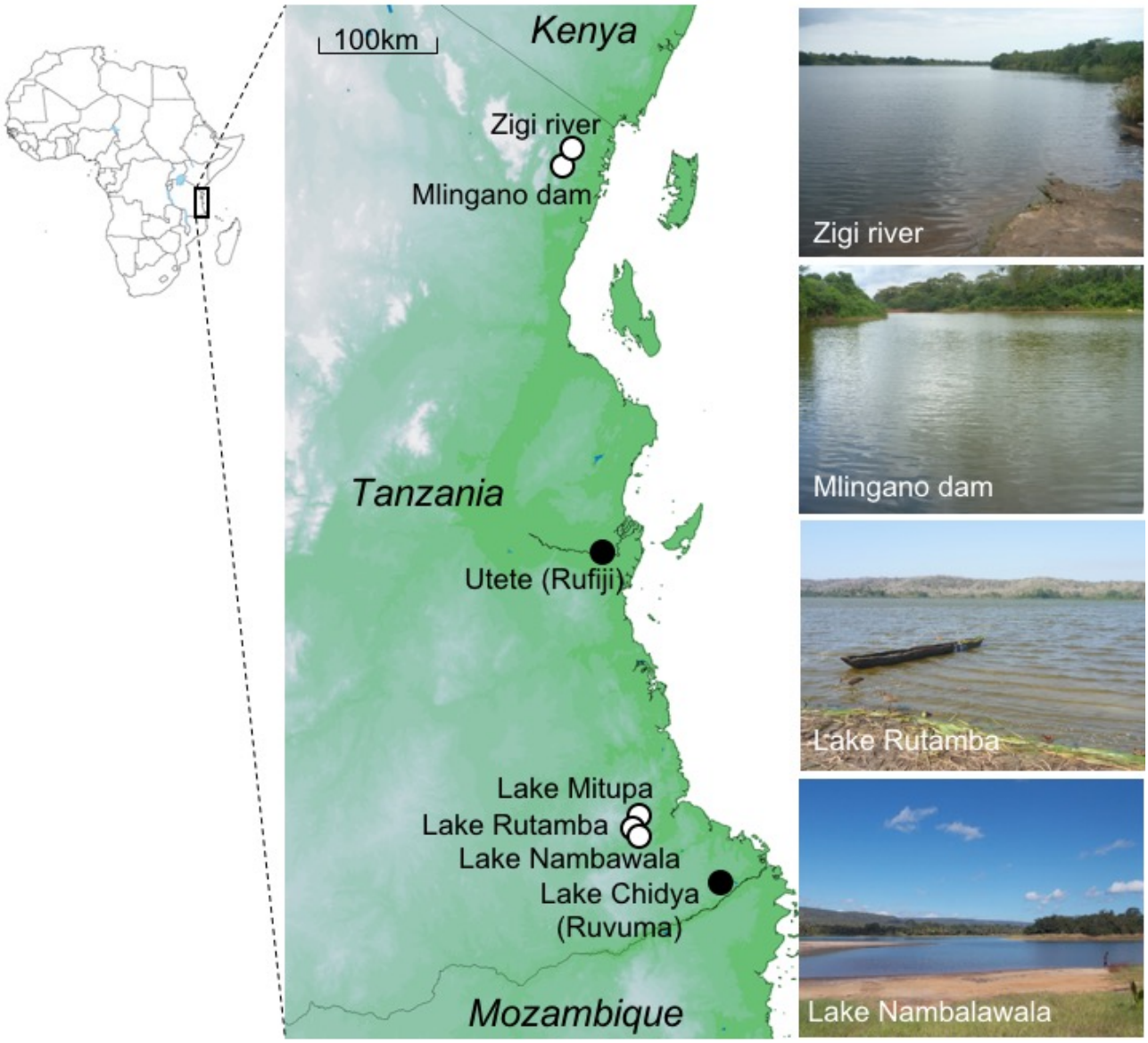

Figure 1. Sampling locations of the four focal sites of *O. korogwe* in Tanzania. Filled circles show sites where reference populations of *O. placidus* and *O. urolepis* were collected.

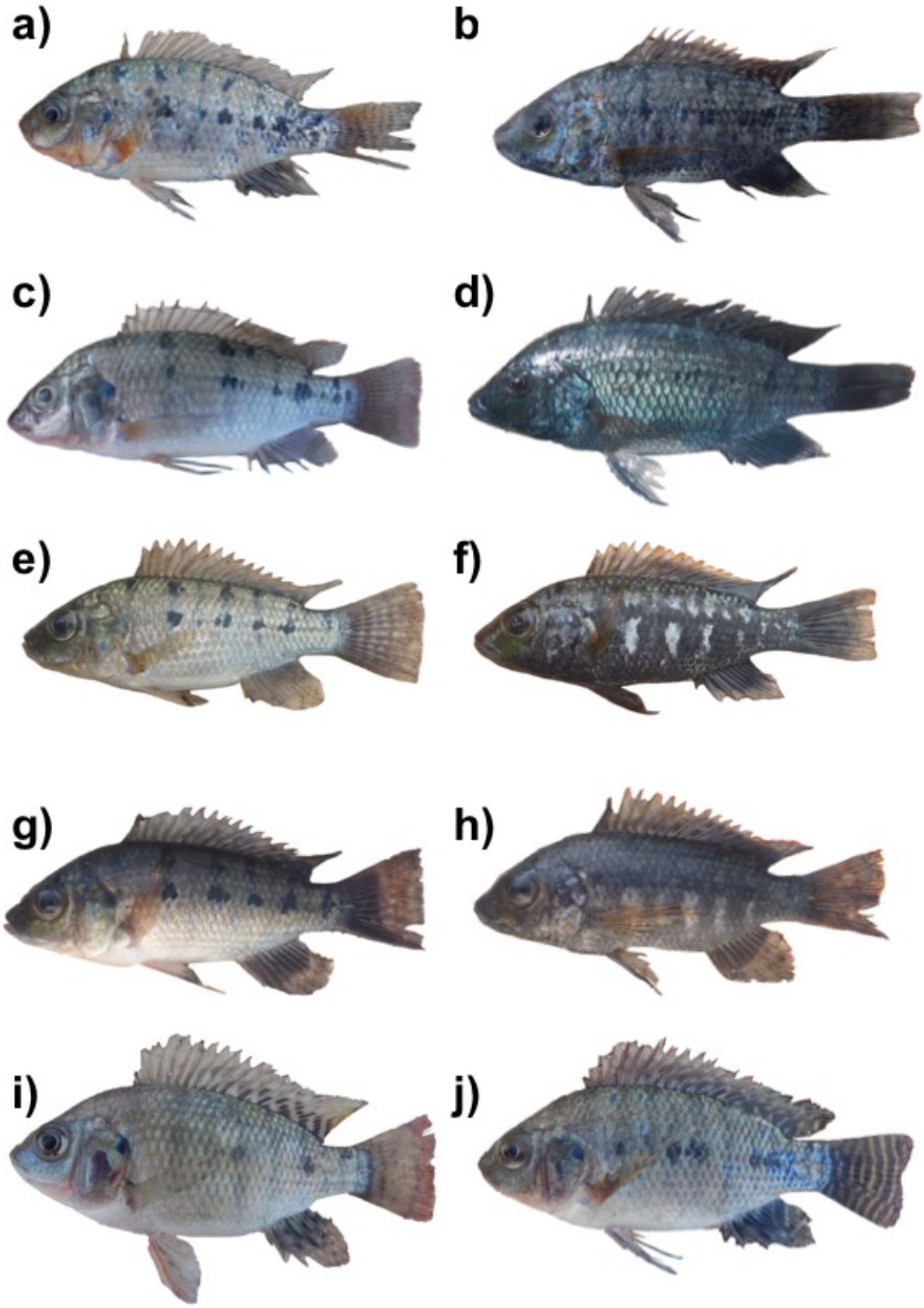

Figure 2. Specimens of *O. korogwe* used in sampling from southern and northern sampling locations in Tanzania a) Rutamba *O. korogwe* female, b) Rutamba *O. korogwe* male, c) Nambawala *O. korogwe* female, d) Nambawala *O. korogwe* male, e) Mlingano dam *O. korogwe* female, f) Mlingano dam *O.*

korogwe male, g) Zigi river *O. korogwe* female, h) Zigi river *O. korogwe* male, i) Rutamba *O. niloticus* female, j) Rutamba hybrid (OK v ON) female.

On collection, fin clips were then taken and preserved in absolute ethanol. For later use in geometric and traditional (linear) morphometrics analyses, each specimen collected in 2016 was placed onto a polystyrene board, individually labelled, fins pinned out, and allowed to fix into position for later morphometrics. The whole specimen was preserved in 100% ethanol prior to later long-term preservation in 70% ethanol.

For comparative genetic analyses, we also included ethanol preserved fin-clip samples of *O. urolepis* from Utete on the Rufiji river sampled between on 11 March 2015 and 29 April 2015, and *O. placidus rovumae* from Lake Chidya in the Rovuma catchment sampled on 18 August 2013 (Fig. 1).

Microsatellite genotyping

DNA was extracted from fin clips using the Wizard kit from Promega (Madison, WI). We genotyped samples at 13 microsatellite loci (Supplementary Information Table S2), sourced from Saju *et al*. (2010) and Liu *et al.* (2013). Polymerase Chain Reactions were performed in 10μl solutions including: 1μl DNA, 0.2μl of each 10μM forward primer, 0.2μl of each 10μM reverse primer, 5μl 2x Qiagen Multiplex PCR Master Mix, the remainder was RNase-free water. PCR was conducted on a Prime thermocycler (Techne), with the following settings: an initial denaturation at 95°C for 1 minute, followed by 35 cycles of 94°C 30 seconds, 57°C for 90 seconds, 72°C for 60 seconds. The final extension stage was 60°C for 30 minutes. Products were genotyped on Applied Biosystems 3500 Genetic Analyser alongside a LIZ500 size standard, and peak sizes were scored using the software Genemapper 4.1 (Applied Biosystems; CA). To summarize genetic diversity of populations and test for deviations from Hardy Weinberg Equilibrium, we used Arlequin 3.5 (Excoffier and Lischer, 2010).

Hybridization in southern lakes

Hybrid individuals between *O. korogwe* and *O. niloticus* were identified from microsatellite data using a two-step process. 1) For all three lakes simultaneously, we applied the find.clusters function in the R package "adegenet", selecting max.n.clust = 40, and the maximum number of Principal Components, to make a preliminary assignment of individuals to two genetic clusters (K = 2), representing *O. korogwe* and *O. niloticus.* 2) We used Structure 2.3.4 (Pritchard *et al.* 2000) to

quantify probability of assignment of individuals to the two species. Structure runs used K = 2 with the find.clusters assignments as a prior. We used the admixture model, with each run including 100,000 steps as burn-in, followed by 100,000 sampled steps. Runs were repeated a total of 10 times, and Structure results were summarized across the runs using Clumpak (Kopelman *et al.* 2015), with putatively purebred individuals identified as those possessing > 0.9 probability of belonging to either *O. korogwe* or *O. niloticus*, and the remainder considered to be *O. niloticus* x *korogwe* hybrids. To ordinate the genetic structure present within the southern lakes, we used a Factorial Correspondence Analysis in Genetix 4.05 (Belkhir *et al.* 1999).

Genetic structure among Oreochromis species

We compared the genetic structure of the southern *O. korogwe* populations (Lake Nambawala and Lake Rutamba) to the northern *O. korogwe* populations (Zigi River and Mlingano Dam), as well as *O. placidus* (Lake Chidya) and *O. urolepis* (Rufiji river at Utete). Individuals of *O. korogwe* from Lake Mitupa were not included in the analysis due to the small sample size of purebred individuals (n = 6). To assess population genetic structure, we used Structure 2.3.4 (Pritchard *et al.* 2000), using sampling location as a prior. We used the admixture model, with each run including 100,000 steps as burn-in, followed by 100,000 sampled steps. Runs for each potential number of clusters *K* (between 2 and 6), were repeated a total of 10 times, and the results were summarized using Clumpak (Kopelman *et al.* 2015). Within Clumpak the Evanno method (Evanno *et al.* 2005) was used to identify the optimal number of clusters present in the data. To ordinate the genetic structure present within the Rutamba lakes region, we used a Factorial Correspondence Analysis in Genetix 4.05 (Belkhir *et al.* 1999). Genetic structure among the populations was estimated in Genepop 4.2 (Rousset, 2008) using F_{ST} and the significance of differences among populations was estimated using Exact tests with default settings.

Geometric morphometrics of Oreochromis species and hybrids from southern lakes.

Ethanol preserved specimens (collected in 2016) were photographed on their left side in standard orientation. The image was calibrated for size and 24 landmarks (Supplementary information Fig. S1) were placed onto the image of each specimen using tpsDIG 1.40 (Rohlf, 2004). All genotyped fish (collected in 2016) were included in geometric morphometrics, except for 7 specimens of *O. korogwe* from Lake Rutamba where pelvic fins were absent. Landmark data were subjected to a Procrustes analysis in MorphoJ 1.06 (Klingenberg, 2011). Individuals were then assigned to one of three groups based on Structure results (pure *O. niloticus,* pure *O. korogwe*, hybrid *O. niloticus* x *korogwe*). The Procrustes coordinates were then subjected to a pooled within-group regression against

centroid size in MorphoJ 1.06. Size standardized residuals from this regression analysis were then used in a stepwise Discriminant Analysis in SPSS 24 (IBM, London), with pure *O. niloticus and* pure *O. korogwe* placed in *a-priori* known categories, and hybrid individuals remaining uncategorized.

Traditional morphometrics of Oreochromis species and hybrids from southern lakes

Linear morphometric measurements were taken of each genotyped specimen (collected in 2016) using digital calipers, following methods outlined in Barel *et al.* (1977), Snoeks (2004) and Zengeya *et al.* (2011). The following measures were made for each individual: standard length, body depth, head length, caudal peduncle length, caudal peduncle depth, dorsal fin base length, anal fin base length, pectoral fin base length, pelvic fin length, caudal fin length, head width, snout length, eye length, interorbital width and lower jaw length. Measurements were $\log_{10}$ transformed, individuals were assigned to the three different groups based on Structure results (pure *O. niloticus,* pure *O. korogwe*, hybrid *O. niloticus* x *korogwe*) and we then conducted a pooled-within groups regression of each variable against standard length. Size standardised residuals from these regressions were then used in a Discriminant Analysis in SPSS 24, with pure *O. niloticus and* pure *O. korogwe* placed in *a-priori* known categories, and hybrid individuals remaining uncategorized.

Morphological comparisons of northern and southern O. korogwe

We compared the morphology of genetically purebred individuals of *O. korogwe* from Lakes Rutamba and Nambawala to individuals from the Mlingano Dam and Zigi River in northern Tanzania. We collected the same geometric morphometric landmark and linear morphometric measurement data for each individual using the methods as described above. The geometric morphometric landmarks were used in a Procrustes analysis and the resultant Procrustes coordinates were subjected to a pooled within-group regression against centroid size, generating size standardized residuals. These residuals were used in a Canonical Variates Analysis in MorphoJ 1.06, and a Discriminant Analysis in SPSS 24. Linear morphometric measurements were $\log_{10}$ transformed, we then conducted a pooled-within groups regression of each variable against standard length, treating each of the four populations as a group. The size-standardized residuals generated from these regressions were then used in used in a Discriminant Analysis in SPSS 24.

Results

Genetic characterization of purebred and hybrid Oreochromis in southern lakes

Structure analysis demonstrated that most individuals were able to be assigned with confidence to one of the two parent species (>90% probability of assignment), while 29% of individuals were interpreted as hybrids in Lake Mitupa (2 of 7), 27% as hybrids in Lake Nambawala (6 of 22), and 6% in Lake Rutamba (2 of 32) (Fig. 3). A Factorial Correspondence Analysis ordination plot supported these results, indicating *O. korogwe* and *O. niloticus* to be genetically distinct, while hybrid *O. niloticus* x *korogwe* were placed between individuals of the parental species (Fig. 3).

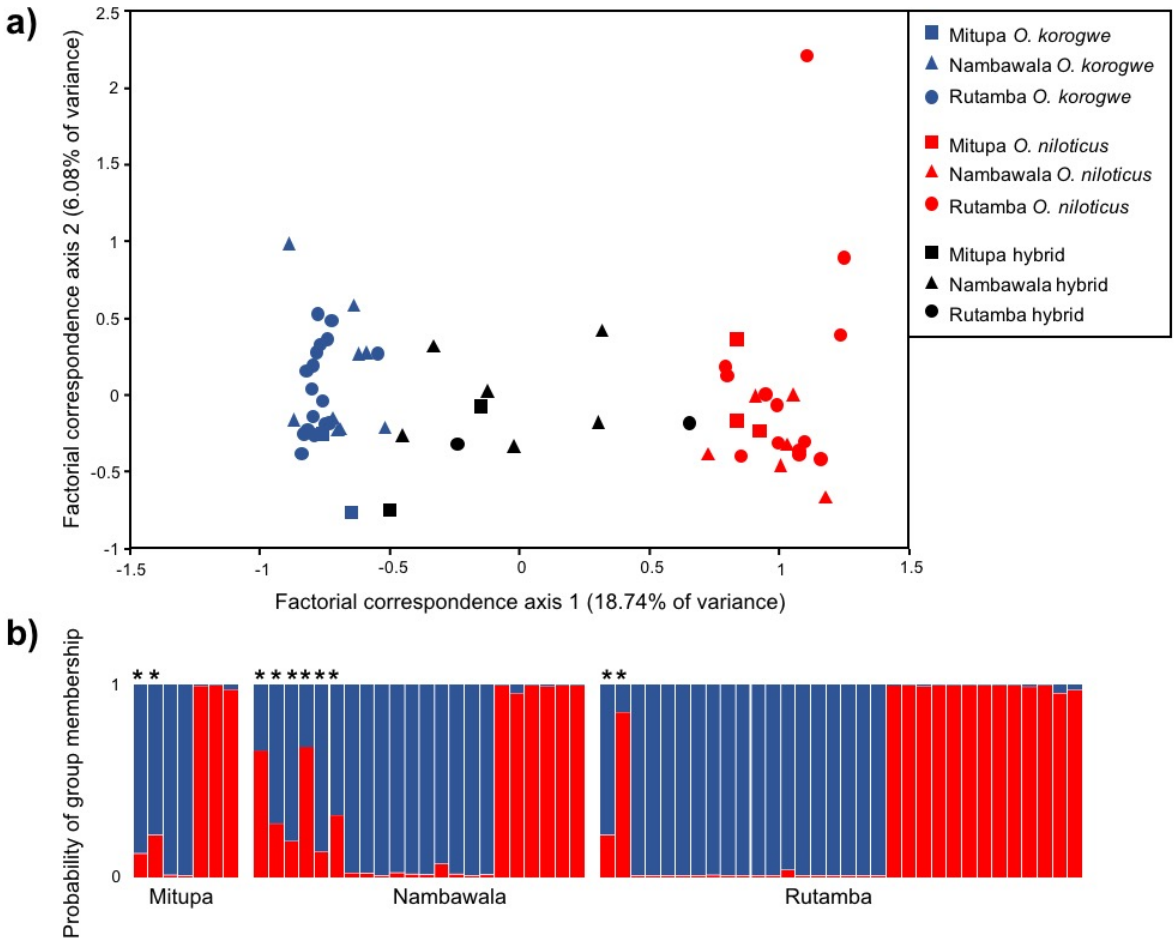

Figure 3. a) Factorial Correspondence Analysis (FCA) and b) Structure analysis of genetic data from *Oreochromis* from the southern lakes. *indicates individuals resolved as of hybrid origin.

Morphological comparisons of Oreochromis from southern lakes.

Discriminant Analysis demonstrated that parental species could be reliably separated using geometric morphometric data (Wilk's $\lambda = 0.272$, $\chi^2 = 37.054$, $P < 0.001$) with 30 of 32 purebred individuals

correctly classified (Table 3). Similarly, there was an overall significant difference between the species using Discriminant Analysis of Traditional linear morphometric measurements (Wilk's λ = 0.314, χ^2 = 32.401, P <0.001), with *O. niloticus* being characterized by a longer and broader head (Table 4). Using these linear morphometric measurements 29 of 32 purebred individuals were correctly classified (Table 3). Overall, hybrids were not readily distinguishable from parental populations using either dataset (Fig. 4; Supplementary Information Table S2).

Table 3. Original and predicted group membership results from Discriminant function analysis of *O. korogwe*, *O. niloticus* and identified hybrids in the southern lakes, using traditional methods and geometric morphometric analysis.

		Classified group		
Measurements	Original group	*O. korogwe*	*O. niloticus*	Total
Linear (traditional)	*O. korogwe*	16	2	18
	O. niloticus	1	13	14
	Hybrids (OK x ON)	6	2	8
Geometric morphometric	*O. korogwe*	17	1	18
	O. niloticus	1	13	14
	Hybrids (OK x ON)	4	4	8

Table 4. Correlation of traits with Discriminant Function Axis 1 separating *O. niloticus* from *O. korogwe* in the southern lakes, using linear (traditional) measurements. Bold indicates the variables with the strongest association with Axis 1.

Trait	Correlation with Axis 1
Head Width	**0.533**
Head Length	**0.392**
Anal fin base length	-0.370
Eye length	0.367
Body depth	0.205
Inter orbital width	0.192
Pelvic fin length	0.165
Caudal fin length	-0.120
Caudal peduncle length	-0.115
Pectoral fin base length	-0.105
Snout length	-0.080
Dorsal fin base length	-0.058
Caudal peduncle depth	-0.050
Lower Jaw length	-0.013

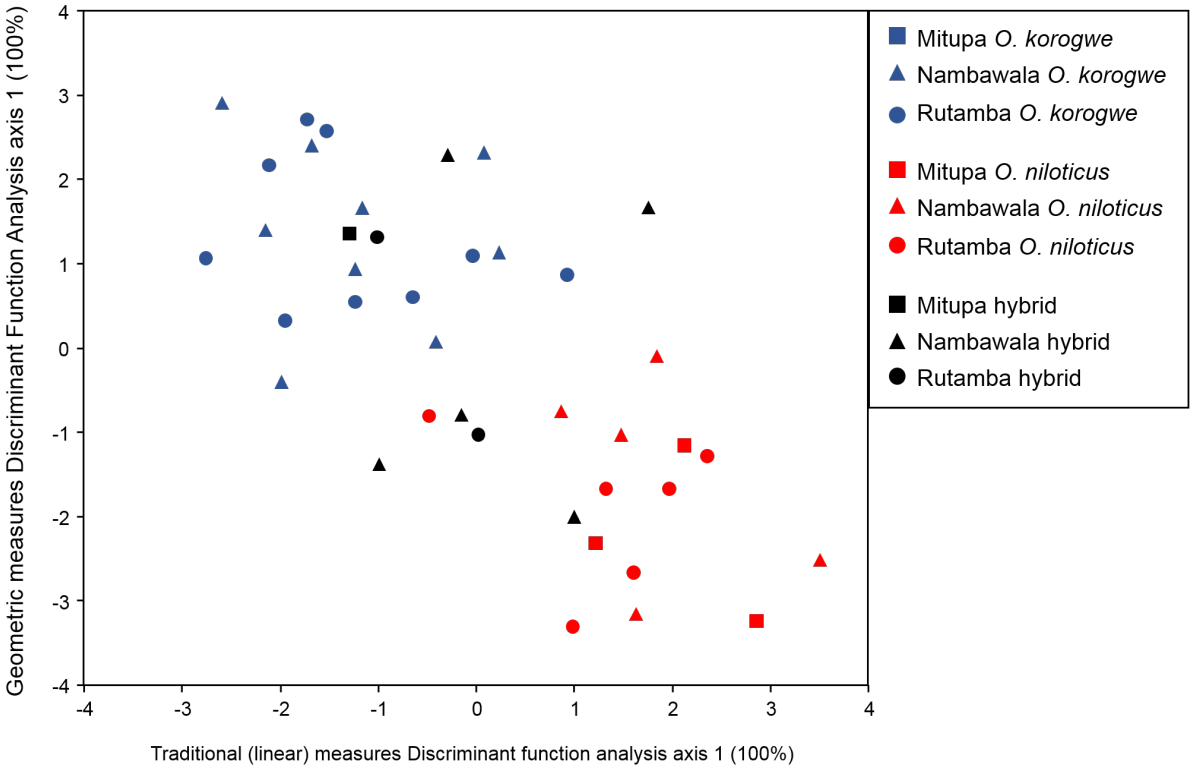

Figure 4. Discriminant function analysis of *Oreochromis* morphology from Lakes Nambawala, Rutamba, and Mitupa, using data from Linear (traditional) measures (x-axis) methods against geometric morphometric analysis (y-axis). Analyses were trained on individuals identified as purebred, while hybrid individuals were left unclassified.

Genetic diversity of Oreochromis populations

Populations showed no clear patterns of significant deviation from Hardy-Weinberg Equilibrium (Supplementary Information Table S1). *Oreochromis korogwe* from the Mlingano dam only deviated significantly from expectations at one of six polymorphic loci tested, while *Oreochromis korogwe* from the Zigi River deviated significantly from expectations at two of ten loci tested. Maintaining only purebred individuals, *Oreochromis korogwe* from Lake Rutamba deviated significantly from expectations at six of 11 loci, while *Oreochromis korogwe* from Lake Nambawala deviated significantly from expectations at only one of 11 loci. *Oreochromis placidus* from Lake Chidya deviated significantly from Hardy-Weinberg Equilibrium at two of nine loci, while *Oreochromis urolepis* from Utete deviated significantly from Hardy-Weinberg Equilibrium at three of 13 loci tested. All significant deviations accompanied lower levels of heterozygosity than expected (Supplementary Information Table S1).

Genetic structure among Oreochromis species

Factorial Correspondence Analysis showed all *O. korogwe* were distinct from reference populations of *O. urolepis* from the Rufiji river and *O. placidus* from Lake Chidya (Fig. 5). Separate analysis of *O. korogwe* populations revealed the Zigi river and Mlingano dam populations to be distinct from one another, and to both populations from the south (Fig. 5). Meanwhile, *O. korogwe* populations from Lake Rutamba and Lake Nambawala in southern Tanzania showed considerable overlap. Structure analyses indicated the most likely number of populations across the three species was K = 5. This analysis supported the distinctions seen in the FCA, with clear separation *O. urolepis* and *O. placidus* from *O. korogwe*, and within *O. korogwe* populations being distinct except for the geographically proximate Lake Rutamba and Nambawala populations (Fig. 5). In pairwise comparisons all populations showed highly significant genetic differences, with exception of the *O. korogwe* from Lakes Rutamba and Nambawala (Table 5; Supplementary Table S4).

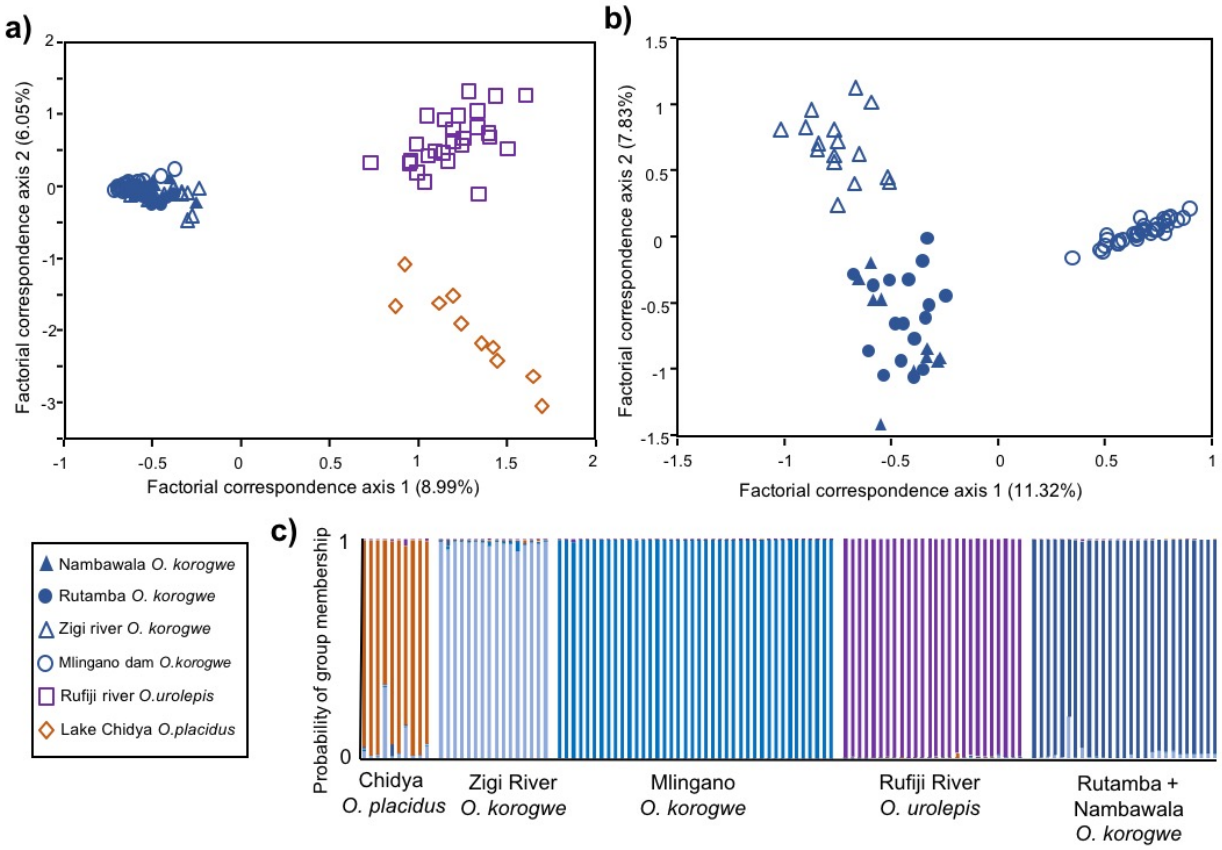

Figure 5. Genetic analysis of populations from northern and southern regions of Tanzania. a) Factorial correspondence analysis of all populations from all six sites, b) Factorial correspondence analysis of the four populations of *O. korogwe* and c) Structure analysis of the six populations, using the most likely number of populations and the Evanno method (K = 5).

Table 5. Matrix of F_{ST} pairwise comparisons (below left) and corresponding *P* values from Exact tests (above right).

	P-C	K-Z	K-M	U-R	K-R	K-N
O. placidus Lake Chidya (P-C)		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
O. korogwe Zigi (K-Z)	0.547		**<0.001**	**<0.001**	**<0.001**	**<0.001**
O. korogwe Mlingano (K-M)	0.761	0.341		**<0.001**	**<0.001**	**<0.001**
O. urolepis Rufiji (U-R)	0.229	0.455	0.612		**<0.001**	**<0.001**
O. korogwe Rutamba (K-R)	0.659	0.358	0.378	0.511		0.473
O. korogwe Nambawala (K-N)	0.618	0.415	0.470	0.461	**0.011**	

Morphological comparisons of northern and southern O. korogwe

Canonical variate analysis of geometric data largely separated *O. korogwe* populations, with the greatest differences between the northern and southern populations along CVA axis 1. (Fig. 6). Wireframe diagrams were indicative of the northern *O. korogwe* populations possessing smaller eyes and narrow body dimensions than southern populations (Fig. 6). Discriminant Function Analysis of both the geometric morphometric data and the traditional morphometric data also demonstrated highly significant differences among the groups on the Discriminant axes (Table 6, Fig. 7), with the majority of individuals being able to be classified reliably into groups using either linear traditional measurement data (74 of 80 individuals), or geometric morphometric data (84 of 88 individuals; Table 7). The southern populations were characterized by deeper bodies, longer caudal fins and larger heads (Table 8; Supplementary Table S2). On average, the southern populations had a larger standard length (SL) with the largest specimen from lake Nambawala measuring 102.68 mm (Supplementary Table 2). DFA axis 2 separated Nambawala and Rutamba fish, with those from Nambawala having greater body depth, caudal fin length, eye length, and head length and width (Table 8).

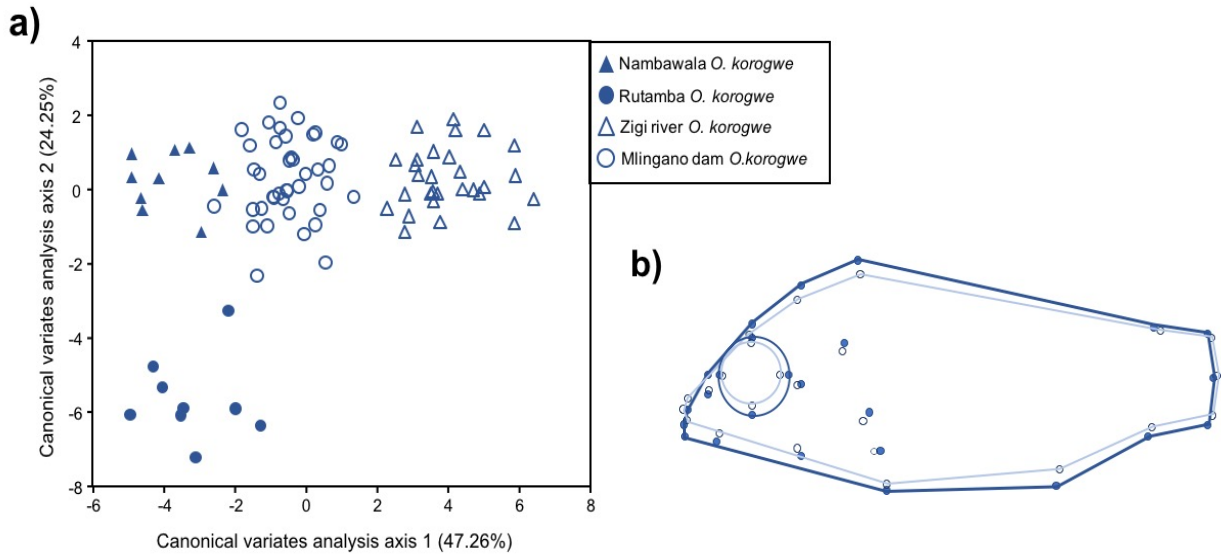

Figure 6. Canonical variate analysis (CVA) of the four populations of *O. korogwe* from northern and southern Tanzania, a) ordination plot of illustrating morphological similarity of individuals, and b) wireframe diagram showing shape variation along CVA axis 1. Filled circles are representative of *O. korogwe* with negative values on CVA axis 1 (e.g. Lake Nambawala), open circles are indicative of positive values (e.g. Zigi river).

Table 6. Morphological differences among southern and northern populations of *O. korogwe*, tested using Discriminant Function Analysis.

Measurements	Test (axes)	Wilk's λ	χ^2	df	P
Linear (traditional)	1 through 3	0.029	253.76	30	< 0.001
	2 through 3	0.188	120.37	18	< 0.001
	3	0.499	50.03	8	< 0.001
Geometric morphometric	1 through 3	0.038	259.52	33	< 0.001
	2 through 3	0.201	127.51	20	< 0.001
	3	0.573	44.30	9	< 0.001

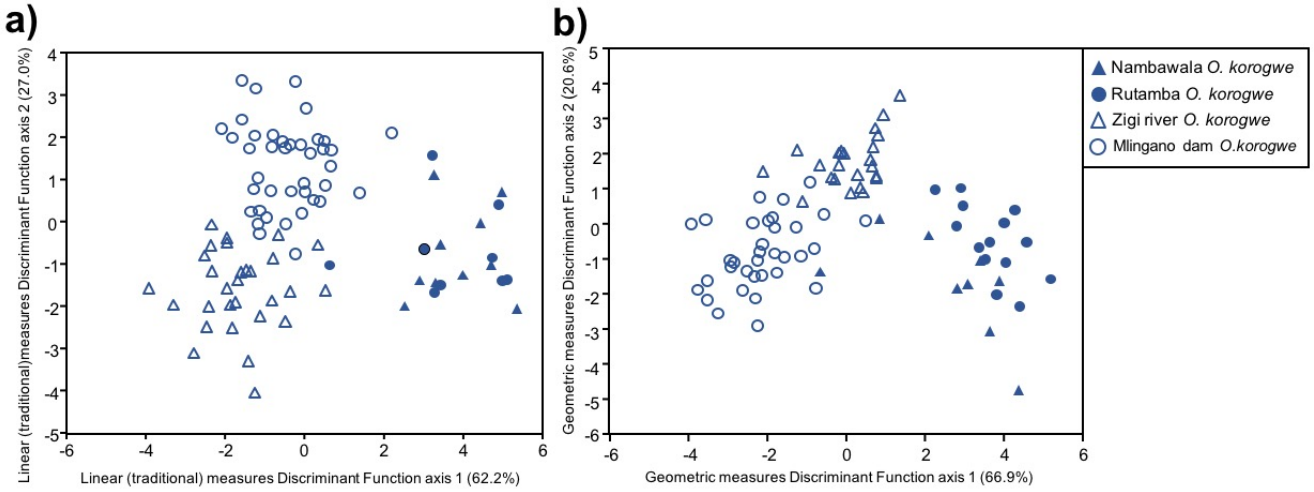

Figure 7. Discriminant Analysis of morphology of *O. korogwe* from two northern and two southern sites, based on data from a) traditional linear measurements and b) geometric morphometrics.

Table 7. Classification results from Discriminant Function Analysis of four populations of *O. korogwe* from a) traditional measures of morphology and b) geometric morphometric measures.

		Classified group				
Measurements	Original group	K-M	K-Z	K-N	K-R	Total
Linear (traditional)	*O. korogwe* Mlingano (K-M)	31	3	0	0	34
	O. korogwe Zigi river (K-Z)	0	23	0	0	23
	O. korogwe Nambawala (K-N)	0	0	13	1	14
	O. korogwe Rutamba (K-R)	1	1	0	7	9
Geometric morphometric	*O. korogwe* Mlingano (K-M)	10	0	0	0	10
	O. korogwe Zigi river (K-Z)	0	8	1	0	9
	O. korogwe Nambawala (K-N)	0	0	28	1	29
	O. korogwe Rutamba (K-R)	0	0	2	38	40

Table 8. Correlation of traits with Discriminant Function axes separating *O. korogwe* populations, using linear (traditional) measurements. Bold indicates variables with the strongest associations with the axes of variation.

Trait	Correlation with DF Axis 1	Correlation with DF Axis 2	Correlation with DF Axis 3
Anal fin base length	0.148	-0.036	-0.045
Body depth	**0.314**	-0.061	0.172
Caudal fin length	-0.090	0.291	**0.828**
Caudal peduncle depth	**0.446**	-0.049	0.157
Caudal peduncle length	0.089	0.133	-0.105
Dorsal fin base length	0.169	-0.050	-0.276
Eye length	-0.197	**0.430**	0.246
Head length	0.030	0.174	**0.500**
Head width	-0.130	-0.019	**0.476**
Inter-orbital width	0.226	**0.458**	**0.337**
Lower jaw length	0.141	-0.086	**0.511**
Pectoral fin length	**0.365**	0.031	0.149
Pelvic fin length	-0.090	**0.470**	0.197
Snout length	0.016	**0.338**	0.318

Discussion

This study confirmed close evolutionary relationship between *O. korogwe* individuals in the northern and southern Tanzania, and their distinctness from populations of two other *Oreochromis* naturally present in coastal rivers of Tanzania, namely *O. placidus rovumae* and *O. urolepis*. Our results also confirmed the presence of hybrids between *O. korogwe* and invasive *O. niloticus* in all three of the southern lakes, with a frequency of hybridization between 7 and 30% of sampled individuals. This level of hybridization is likely to be an underestimate if backcrosses with purebreds are present (Boecklen and Howard, 1997), and further work will be needed to fully establish the extent of hybridization in each of the lakes.

Hybridization commonly occurs following the introduction of non-native species due to the absence of strong reproductive barriers that are typically present among naturally sympatric taxa (Horreo *et al.* 2011, Gainsford, 2014). In many taxa, prezygotic barriers have been suggested to play an important role in the maintenance of hybridization (Mallet, 2005). *Oreochromis* species, like many African mouthbrooding cichlids, show strong sexual dimorphism in communication signals,

suggesting that strong sexual selection may reinforce reproductive isolation and prevent introgression between species (Becher and Gumm, 2018). Little is known about the breeding behaviour of *Oreochromis korogwe* or *O. niloticus* in the study system however, and it is plausible that reproductive isolation is broken down through either female preference for heterospecifics, or because of changes to availability of suitable mates. Typically, *Oreochromis* males are lek-forming and highly territorial, building large spawning bowers within the centre of the territories (Trewavas, 1983). It is possible that the larger *O. niloticus* males have effectively excluded smaller *O. korogwe* males from suitable breeding habitats; detailed survey and experimental work is required to test this hypothesis, including tests of sex-biases in the direction of hybridization (e.g. Hayden *et al.* 2010; Rognon and Guyomard, 2003).

Postzygotic barriers can be important in determining the frequency of hybrids within the natural environment (Wiley *et al.* 2009), through reducing fitness of hybrid progeny. Reduced fitness of hybrids has been recorded in cichlids (Maan *et al.* 2017) and other fishes (Muhlfeld *et al.* 2018). Although *Oreochromis* species are generally known to produce viable and fertile offspring (Rognon and Guyomard, 2003), little is known about the viability and fertility of *O. korogwe* x *niloticus* hybrids. Further work to investigate their fitness in relation to the parental species will be needed to fully assess the extent and impact that these hybridization events will have on the evolution and persistence of these newly discovered *O. korogwe* populations. This will require laboratory mate choice experiments, alongside field studies where *O. korogwe* exist in allopatry versus in sympatry with *O. niloticus*. Research has suggested that post-zygotic mechanisms are often underestimated when looking only at the fitness of F1 hybrids, therefore to accurately investigate this form of isolation, multi-generational research is needed (Wiley *et al.* 2009; Arnold and Hodges, 1995).

Population structure of southern and northern O. korogwe.

There is a 500 km gap between the northern and southern populations of *O. korogwe* in Tanzania. Typically, such gaps are due to human intervention, and stocking has resulted in *O. niloticus* having a broad discontinuous distribution across Africa, and further afield (Guyon *et al.* 2012; Deines *et al.* 2014). However, there is currently no evidence that *O. korogwe* was stocked, either from the north to the south, or vice versa. To our knowledge, the only evidence for *O. korogwe* being associated with aquaculture comes from the original species description (Lowe, 1955), where the collection locality of the type specimens is reported to be the Tanganyika Government experimental fish farm at

Korogwe. However, Lowe-McConnell (2006) later comments that Major R.E, Gould, who ran the facility, had reportedly moved species without governmental permission, and without recording his transfers. Thus, unrecorded movements of fish were apparently taking place during the 1950s, and the stocking of *O. esculentus* from Lake Victoria into the Singida lakes and Lake Rukwa provides ample evidence of long-distance transfer of tilapiine species during the colonial era (Trewavas, 1983). If the southern lakes were stocked from the Pangani system during the 1950s, or later, then we may expect the introduced populations to possess relatively low allelic diversity (e.g. Ambali *et al*. 1999). However, we saw no clear evidence for lower genetic diversity in the southern populations. It is plausible these are natural populations of *O. korogwe,* the result of either a natural long-distance colonization event, or perhaps that the species once had a wider distribution that has been disrupted through either extirpation or introgression with *O. urolepis*, a species that neatly fits the gap between the range of *O. korogwe.* To investigate whether this is the case, future work could test for introgression between *O. urolepis* and *O. korogwe* at the boundary regions where these species are found in close proximity. It would also be valuable to calculate the timescale of divergence between *O. korogwe* populations using recently derived estimates of the per generation mutation rate across whole cichlid genomes (Malinsky *et al.* 2017).

Morphological variation among O. korogwe populations

Our results show that the northern and southern populations of *O. korogwe* were largely distinct in characters such as body depth, fin length, eye size and snout length. Based on the data alone, it is not possible to determine the extent that these differences are a result of fixed genetic differences, or alternatively differences in the environments during development. Such phenotypic plasticity in morphological traits is well known in cichlids, and in part have helped to contribute to the rapid diversification of some cichlid lineages (Parsons *et al.* 2011; Schneider and Meyer, 2017). Irrespective of whether the variation is largely genetic or plastic, it is plausible that the differences are functional, as differences in head and body shape are often related to resource use patterns in cichlids. For example, variation in jaw morphologies is linked with feeding habits (Burress, 2015), while eye size is related to visual environment (Hahn *et al.* 2017), and fin morphology is related to patterns of habitat use (Colombo *et al.* 2016). Little is known about the feeding habits of *O. korogwe* (Dieleman *et al.* 2015), and detailed analysis of diets and foraging environments within the sampled locations would be required to explore functions of morphological variation observed. To determine the extent that additive genetic variation or phenotypic plasticity contribute to the variation in this

species, a common garden experiment across generations would be an important next step (e.g. Rader *et al*. 2005; Belk and Schaalje, 2016).

Conservation implications

Our results are not sufficiently conclusive from either genetic or morphological perspectives to warrant spitting the northern and southern populations into discrete species. Further genome-level analyses coupled with detailed morphological analyses and laboratory mate choice experiments would help us to determine if description of the southern populations as a new species is appropriate. Nevertheless, our results are suggestive of both the northern and southern populations representing unique sets of genetic resources, which will have implications for the biodiversity of tilapias of East Africa. Such populations can be considered to represent evolutionary significant units (ESUs), a measure that can be useful where taxonomical distinction is subjective (e.g. Belk and Schaalje, 2016; Hallerman and Hilsdorf, 2014).

Our results illustrate that genetic structure within the newly discovered populations of *O. korogwe* was very likely already being impacted by the non-native alien species *O. niloticus*, even before their first formal recognition in 2013. Species introductions are one of the most non-reversible impacts on genetic diversity (Dudgeon *et al.* 2006), and therefore the presence of this highly invasive species in these lakes is of considerable concern for the long-term viability for these populations. Hybridization, while currently reported at a relatively low intensity in these southern lakes, could have larger impacts on the genetic diversity of this population over time, especially given evidence from other lakes where *O. niloticus* have been introduced (e.g. Deines *et al.* 2014), and given the lack of understanding of the long-term fitness consequences of these interactions (Wiley *et al.* 2009). Although there is some evidence that hybridization could introduce advantageous alleles into the population, our findings suggest that these southern populations are likely to be locally adapted to the southern lakes, and therefore introgression is likely to have negative outcomes for the genetic uniqueness of the *O. korogwe* populations at least.

Although *O. niloticus* will have been introduced to the southern lakes as a measure to boost fisheries production, hybridization could have implications for the long-term viability of the local fishery, both in term of productivity and profitability. Hybridization between the large bodied *O. niloticus* and small bodied *O. korogwe* could lead to a substantial reduction in production in these areas due to the

production of smaller hybrid individuals dominating the population. In addition, to fully understand the impact these *O. niloticus* will have native species, it would be important to investigate whether the two species overlap significantly in their niches, and whether competition could be another threat to the survival of these newly discovered *O. korogwe* populations.

Given the removal of *O. niloticus* from the southern lakes would be impractical, conservation of the unique genetic resources within the southern lakes would likely be best done through the identification of potential ark sites, long-term monitoring of the genetic and phenotypic diversity within the studied lakes, and potentially *ex-situ* conservation. We have only sampled three of the water bodies in close proximity to the towns of Lindi and Rutamba, and it is possible that *O. korogwe* populations unaffected by *O. niloticus* are present in other proximate water bodies. Further exploration of the fish biodiversity of southern Tanzania would be invaluable for freshwater conservation planning in the region.

Acknowledgements for Paper Chapter

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Chapter 3: Research Summary

Our results showed clear genetic and morphological distinctions between the northern populations and the newly discovered southern populations of *Oreochromis korogwe*. We concluded that these populations present unique genetic resources that are already being threatened by hybridization with the non-native invasive species *Oreochromis niloticus.* This work brings up several key questions that need further investigation.

The first question is what the true distribution of these distinct *O. korogwe* is across Tanzania. If these new populations originated from the Pangani and Zigi river systems, or from the Korogwe ponds, more than 500 vbdcfERkm north, it is likely that their presence may not be restricted to these three southern lakes, particularly given the scarcity of data on these smaller sites across Tanzania. Investigation of lakes in the surrounding area will need to be conducted in order to establish the full extent of their occurrence in Tanzania. In order for their occurrence to be accurately measured, a more comprehensive definition of their defining phenotypes is needed.

Whilst investigating the surrounding lakes in the Rutamba region, it would be prudent to also establish the extent of hybridization occurring, as it is likely that *Oreochromis niloticus* have also spread further than previously thought. Recent research has highlighted that suitable habitat for *Oreochromis niloticus* is likely to expand (Shechonge *et al.* 2018), therefore the extent of hybridization in this region may be larger than estimated here. If these populations of *O. korogwe* are distinct to the northern populations, they present unique genetic resources that are at threat, and understanding the extent of hybridization will be crucial to establishing the impact this invasive species is having in this region. Further genetic work should also be conducted to establish whether any 'pure' populations of *O. korogwe* are still present in the southern region.

Whole genome sequencing of cichlid fishes has been conducted to estimate the rate of mutation to understand the genomic processes promoting rapid cichlid adaptive radiation (Malinsky *et al.* 2017). These estimated mutation rates could allow us to accurately analyse the demography of these northern and southern populations of *O. korogwe*, including the timing of their divergence. Future work can now be conducted to include a more comprehensive sequencing of the genome of both populations, along with reference populations of *O. niloticus*, *O. placidus*, and *O. urolepis,* in order to fully understand the genetic relationships between these species.

Along with investigating the extent of hybridization, further work to estimate the outcome of these events is needed. It will be important to assess whether hybrid progeny display reduced or increased fitness (Feurtey *et al.* 2017). The use of controlled crosses to investigate the viability and fecundity of hybrid progeny could give vital information on the outcome of these hybridization events (Arnold and Hodges, 1995). In order to understand the effect these hybridization events will have on the long-term persistence of this newly discovered species, these experiments should span several generations of hybrids as fitness has often been shown to vary between F1, F2, backcrosses and later generation hybrids (e.g. Wiley *et al.* 2009).

The mechanisms by which hybridization occurs, and which allow hybrid progeny to co-exist with parental species are not well understood (Hayden *et al.* 2011). Life history traits have often been suggested to provide reproductive barriers to hybridization (Hayden *et al.* 2010), however, relatively little is known about the life history of *Oreochromis korogwe* (Trewavas, 1983). The use of stable isotope analysis could give some information on the ecological niches held by 'pure' *O. korogwe* individuals, *O. niloticus* individuals, and their hybrid progeny (Hayden *et al.* 2011), in order to understand how they co-exist. *Oreochromis niloticus* often exhibits broad niche overlap with native congenerics (Zengeya *et al.* 2015), therefore it is likely that hybrids between *O. korogwe* and *O. niloticus* show some overlap in their ecological niches, which could have important implications for the outcome of these hybridization events.

Another next step for this research will be the analysis of mtDNA of these individuals to determine the direction of hybridization, as this has been useful in previous research in this area (e.g. D'Amato *et al*. 2007). Hybridization is likely to be occurring due to a break down in reproductive barriers, such as mate choice (Becher and Gumm, 2018). In order to determine whether it is *O. korogwe* females interbreeding with *O. niloticus* males the analysis of mtDNA will be key. Mate choice experiments to determine the behavioural aspects of these events will also be useful, alongside ecological investigation of these lakes, as this has been found to be an important aspect of mate choice and species recognition among cichlids (Genner *et al.* 2007).

East Africa is well known for the diverse species flocks of Cichlid fishes found in the three Great Lakes (Victoria, Malawi, and Tanganyika), and an extensive amount of research has been conducted on these sites in the past few decades (Salzburger *et al.* 2014). However, relatively little research has focused on smaller lake and river systems around Africa (Dieleman *et al.* 2015). In the event of large scale extinction events, such as that of *Oreochromis esculentus* and *O. variabilis* in Lake Victoria due

to the introduction of the Nile tilapia (*O. niloticus*) and the Nile perch (*Lates niloticus;* Ogutu-Ohwayo, 1990), satellite lakes have been shown to provide important refuges for the unique genetic diversity of this species (Angienda *et al.* 2011).

While *Oreochromis* species are widely used in aquaculture and are important fisheries species (Soler *et al.* 2010), it has often been suggested that the region has an over-reliance on non-native alien species for these purposes (Lind *et al.* 2012a), ignoring native species such as *Oreochromis korogwe* and large bodied species such as *O. urolepis*. The current work focuses on a series of small lakes and river systems across Tanzania, investigating the genetic and morphological variation of several native *Oreochromis* species, as well as the invasive alien species *Oreochromis niloticus*. Understanding the distribution of these species, and the extent of hybridization with invasive species, will provide an important first step for planning conservation strategies (Olden *et al.* 2010). As it is difficult to reverse the impacts of non-native alien species, and hybridization events, the creation of alien and hybrid-free zones is a much more realistic strategy to put in place (Sales *et al.* 2018). Future work to investigate surrounding lakes for the presence of pure *O. korogwe* will therefore be an essential part of the conservation of this species.

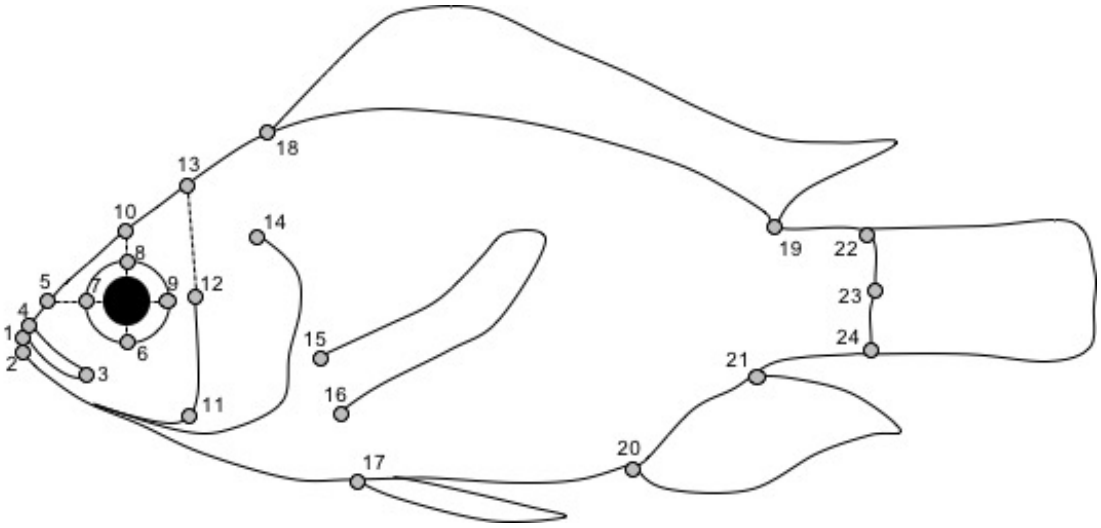
One conservation strategy proposed to reduce the spread of invasive alien species in Africa, is the zoning of aquacultural practices (Darwall *et al.* 2005; Lind *et al.* 2012a). This strategy suggests that aquacultural and fisheries practices use only species that are native to each particular region (Lind *et al.* 2012a). The success of this strategy would clearly require knowledge of native species distributions. The recent discovery of *O. korogwe* in southern Tanzania highlights the importance for further work on smaller lake and river systems. These populations may provide a previously untapped resource to increase the production for aquaculture and fisheries in the region without increasing the spread of other non-native species, therefore the appropriate management of these populations may be crucial for the economy of the area. They may also present a source of genetic diversity for the use in genetic improvement programmes for aquaculture (Brummer *et al*. 2011; Deines *et al.* 2014).

While it cannot be confirmed presently if the population of southern *O. korogwe* are a distinct species, this research did identify clear genetic differences, suggesting that these populations should be managed as a distinct conservation unit to that of the northern populations. The fact that these resources are already being threatened by hybridization with the invasive species *O. niloticus* highlights the need for conservation management in this area. In these small local communities both *Oreochromis korogwe* and *Oreochromis niloticus* are fished, sold, and consumed. While *O. niloticus*

is the larger species of the two and presently makes up a large proportion of production in Lakes Rutamba and Nambawala, their presence may result in the loss of *O. korogwe* from these sites over time, therefore without proper management this invasive alien species could cause large declines in production in these regions, as well as drive biodiversity loss.

Appendices:

Supplementary Information Figure 1. Landmarks used in the geometric morphometric analysis.



Supplementary Information Table S1. Genetic diversity of the focal populations of *O. korogwe*, *O. urolepis* and *O. placidus*. N - number of individuals, NA -number alleles, Ho - Observed heterozygosity, He - Expected heterozygosity, P - probability of Hardy Weinberg Equilibrium.

Site	Species		OMO219	OMO229	OMO337	OMO391	OMO392	OMO397	OMO09	OMO043	OMO129	OMO03	OMO04	OMO01	OMO114
Mlingano	*O. korogwe*	N	33	-	-	-	40	40	-	-	-	-	35	35	40
		NA	66				80	80					70	70	80
		Ho	0.21	-	-	-	0.45	0.58	-	-	-	-	0.83	0.71	0.33
		He	0.25	-	-	-	0.38	0.63	-	-	-	-	0.82	0.57	0.28
		P	**0.03**	-	-	-	0.6	0.45	-	-	-	-	0.99	0.21	0.56
Zigi River	*O. korogwe*	N	12	16	-	15	9	16	16	16	-	5	-	14	14
		NA	24	32		30	18	32	32	32		10		28	28
		Ho	0.17	0.19	-	0.8	0.33	0.81	0.13	0.13	-	0	-	0.07	0
		He	0.16	0.28	-	0.65	0.54	0.66	0.31	0.23	-	0.8	-	0.47	0.14
		P	1	0.05	-	0.82	0.17	0.5	0.05	0.19	-	**<0.001**	-	**<0.001**	0.04
Lake Chidya	*O. placidus*	N	8	10	10	10	-	9	5	-	10	10	10	-	-
		NA	16	20	20	20		18	10		20	20	20		
		Ho	0.13	0.9	0.3	0.1	-	0.56	0.2	-	0.7	0.3	0.7	-	-
		He	0.13	0.73	0.43	0.1	-	0.66	0.87	-	0.62	0.94	0.62	-	-
		P	1	0.73	0.09	1	-	0.21	**<0.001**	-	0.77	**<0.001**	0.77	-	-
Rufiji	*O. urolepis*	N	26	25	26	26	26	26	22	25	26	21	19	22	25
		NA	52	50	52	52	52	52	44	50	52	42	38	44	50
		Ho	0.77	0.84	0.27	0.54	0.35	0.77	0.86	0.52	0.31	0.52	0.74	0.73	0.84
		He	0.79	0.79	0.67	0.69	0.3	0.81	0.88	0.7	0.28	0.92	0.96	0.9	0.77
		P	0.42	0.47	**<0.001**	0.22	1	0.46	0.44	0.03	1	**<0.001**	**<0.01**	**0.05**	0.6
Rutamba	*O. korogwe*	N	16	-	17	17	11	17	16	-	17	8	17	13	13
		NA	2	1	2	2	3	3	2	1	2	5	2	5	2
		Ho	0.19	-	0	0.12	0.45	0.29	0.25	-	0	0.13	0.06	0.15	0.62
		He	0.5	-	0.11	0.11	0.65	0.27	0.31	-	0.51	0.81	0.06	0.63	0.52
		P	**0.03**	-	**0.03**	1	**0.04**	1	0.43	-	**<0.001**	**<0.001**	1	**<0.001**	0.6
Rutamba	*O. niloticus*	N	12	13	13	-	12	12	12	-	-	11	13	6	8
		NA	4	2	2	1	4	2	3	1	1	5	2	3	3
		Ho	0.08	0.54	0.08	-	0.5	0.33	0.17	-	-	0.82	0.08	0.17	0.38
		He	0.72	0.51	0.08	-	0.64	0.39	0.65	-	-	0.77	0.08	0.32	0.64
		P	**<0.001**	1	1	-	0.08	1	**<0.001**	-	-	0.36	1	0.09	0.14
Nambawala	*O. korogwe*	N	7	10	10	-	4	10	10	-	9	1	10	4	4
		NA	2	2	2	1	3	2	2	1	2	2	2	3	2
		Ho	0.29	0.3	0	-	0.5	0.2	0.2	-	0	1	0.2	0.25	0.25
		He	0.26	0.27	0.19	-	0.61	0.19	0.34	-	0.47	1	0.19	0.61	0.25
		P	1	1	0.05	-	0.43	1	0.31	-	**<0.001**	1	1	0.14	1
Nambawala	*O. niloticus*	N	6	6	-	-	6	6	6	6	6	6	6	6	6
		NA	2	2	1	1	3	3	2	2	2	2	2	6	4
		Ho	0.33	0.5	-	-	1	0.17	0.33	0.17	0.17	0.17	0.17	0.5	0.5
		He	0.48	0.53	-	-	0.71	0.62	0.3	0.17	0.17	0.17	0.17	0.8	0.79
		P	1	1	-	-	0.58	**0.03**	1	1	1	1	1	0.06	0.32

Supplementary Information Table S2. Morphological variation among populations of O. korogwe, O. niloticus, and hybrids of O. niloticus and O. korogwe from the 5 focal sites across Tanzania. SD here is Standard Deviation.

Site	Species		SL	BD	HL	CPL	CPD	DFBL	AFBL	PFBL	PFL	CFL	HW	SnL	EL	IOW	LJL
Mlingano	*O. korogwe*	Mean	60.44	21.64	20.64	7.75	8.69	34.05	11.17	3.35	17.03	17.07	11.41	6.34	6.63	7.19	6.27
		Max.	73.78	25.21	26.89	9.66	10.80	41.64	14.05	3.84	20.86	20.30	14.22	8.67	8.23	8.73	8.15
		SD	5.96	1.90	2.41	0.81	0.90	3.29	1.26	0.31	2.02	1.73	1.23	1.08	0.71	0.74	1.05
Zigi River	*O. korogwe*	Mean	57.07	20.88	19.83	6.58	8.40	32.65	10.92	3.37	16.88	16.07	10.44	6.23	6.56	7.22	5.70
		Max.	66.84	26.45	23.43	7.93	9.93	39.12	13.76	4.32	22.21	20.40	12.91	7.38	7.57	8.80	7.26
		SD	4.27	1.96	1.50	0.68	0.65	2.84	1.10	0.40	1.96	1.66	0.86	0.56	0.58	0.66	0.67
Mitupa	*OK* x *ON* Hybrid	Mean	71.60	27.26	24.60	10.17	10.70	41.26	13.01	4.40	18.70	16.12	13.14	8.30	7.50	9.07	8.03
		Max.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		SD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mitupa	*O. niloticus*	Mean	75.11	30.70	28.05	9.10	10.43	43.25	12.26	4.30	18.96	21.25	14.85	7.90	8.52	8.38	8.86
		Max.	78.80	31.99	29.46	9.19	10.78	45.90	12.90	4.75	19.97	23.43	15.29	8.48	9.13	8.15	8.60
		SD	3.41	1.40	1.75	0.15	0.33	2.65	1.05	0.54	1.05	1.97	0.64	0.71	0.83	0.23	0.92
Nambawala	*OK* x *ON* Hybrid	Mean	77.83	30.69	9.53	9.53	11.61	46.49	15.20	5.15	20.52	18.50	13.66	7.33	6.83	9.32	6.80
		Max.	111.67	45.73	30.88	15.47	17.82	68.24	22.76	6.95	28.76	26.21	18.93	10.73	8.94	13.53	9.90
		SD	23.81	9.40	7.02	3.61	3.67	13.63	4.49	1.20	5.85	5.34	3.47	2.46	1.79	3.02	1.94
Nambawala	*O. korogwe*	Mean	76.22	28.56	25.05	8.79	11.72	44.85	15.59	4.54	20.80	18.48	13.34	7.90	7.04	9.09	7.63
		Max.	102.68	40.40	34.59	12.36	16.07	62.14	21.12	6.30	28.07	26.70	19.18	13.77	9.86	12.73	12.31
		SD	19.37	7.62	6.55	2.29	3.12	11.36	4.02	1.12	5.47	6.16	3.66	2.94	1.80	2.44	2.38
Nambawala	*O. niloticus*	Mean	150.25	60.52	47.98	17.10	23.07	93.96	31.25	10.00	38.95	35.01	27.00	14.66	10.92	18.07	13.46
		Max.	161.52	63.98	51.93	18.33	24.44	100.99	31.93	10.81	44.24	39.23	28.03	15.60	12.67	20.06	14.55
		SD	7.61	3.65	2.65	1.23	1.49	4.52	0.75	0.63	3.98	3.80	1.40	0.82	1.13	1.41	0.93
Rutamba	*OK* x *ON* Hybrid	Mean	83.94	34.88	29.34	9.56	12.53	50.71	17.22	5.47	21.18	21.96	15.24	8.92	7.75	10.80	9.84
		Max.	97.77	42.09	33.60	11.57	14.48	60.14	20.11	6.59	23.07	23.88	17.16	10.00	9.01	12.82	10.38
		SD	19.57	10.20	6.02	2.85	2.76	13.34	4.09	1.58	2.67	2.72	2.72	1.53	1.78	2.86	0.77
Rutamba	*O. korogwe*	Mean	73.47	28.91	25.69	8.08	11.56	42.64	14.89	4.60	19.74	21.33	13.86	8.58	7.23	9.60	9.07
		Max.	92.36	36.18	29.93	10.46	14.37	55.09	18.82	5.33	22.77	24.45	16.95	10.59	8.39	11.96	10.35
		SD	10.18	3.66	2.96	1.11	1.80	6.54	2.16	0.57	2.85	1.94	1.94	0.98	0.86	1.37	0.91
Rutamba	*O. niloticus*	Mean	83.78	35.02	29.58	10.03	13.00	49.57	16.00	5.72	20.62	21.22	16.65	9.29	8.73	10.94	9.23
		Max.	104.07	42.64	36.29	13.04	16.00	62.57	19.39	7.20	25.04	24.40	20.26	11.17	9.56	13.21	11.58
		SD	18.30	7.72	6.09	2.43	2.64	11.74	2.96	1.26	3.22	3.34	3.48	1.86	1.19	2.05	1.99

Supporting Information Table S3: Microsatellite loci primer sequences and sources.

Marker name	Genbank Accession	Primer sequence (forward)	Primer sequence (reverse)	Motif
OMO043	JX204857	GGGGTCATTCGGTTTATTGGTTAT	AGGGCAGGTCACGGGTTCG	(TTTG)8
OMO093	JX204891	AAGCCCCACATAGACGACCAGAGA	CAGAAACGGTGCCTGTTCCAGAA	(CAT)8
OMO100	JX204895	CCTTCCCCACCACTACCCTCATAA	CCCGCCCACACCTGACGA	(ATT)18
OMO114	JX204905	ACGCCTTAATGCTGCCTTCAAGA	TGATGCTCACCCCGTTCCTCA	(GTT)11
OMO129	JX204914	TTGGCAGGCTAAGTACTATTTCAT	GAGCGAATGGTTGTCTGTCTCT	(CCAT)9
OMO161	JX204924	ACTTTGACAAAAGAAGTGTAACAA	AGGGGAGGAGAAAATAAACTGTAT	(TAA)10
OMO219	JX204964	ATCCCCTTCTTTCCATCCCTGTC	AAGGCCTCTGTGAGCTGATTGATT	(TTTTG)10
OMO229	JX204973	GCGACTTTTTCTTTGCACATTTTT	AACTGAACCGCCATCATAATCATC	(GTT)9
OMO248	JX204987	AAAGACACAAAGAGAAACTAATCA	GGATGAATATTTAAAATCAGTCAG	(TCA)9
OMO337	JX205052	TAGGAGAGGCATAGGTTGTCAAAT	CAAGAGTCTAGGAGGGAATCAAAA	(GTTT)7
OMO361	JX205069	TGACAGCGAGCCAGAATGGAAGTA	AAAAGTGAAAGGGGCACAGTGAGG	(CTT)17
OMO391	GR699257	AGACATCTGTACGCTCTTTACGAA	AGTGCTAGAGGGAAGGGGCTGTA	(GAT)9
OMO392	GR698887	CTGGCTTAACTTCTCTACTGGACA	TCTACTCAAAACTGGCAACAAAAC	(GAATA)7
OMO397	GR693794	ACGCGTGTTTGAGATATTTAGATT	GAACAAACAAGGGGAGTGG	(GATT)7
OM-01	GU391020	TTTAAAGTTACACAGCAGTACAAAG	TTGTAGCATTTCAACACAGTCTC	(GT)20
OM-03	GU391022	CTTTTTAATGAGCAACTTTTAAGTC	TGTGAATTTGACAACTTCCTTTC	(GATA)47
OM-04	GU391022	AGCTCAAAACCTCATACAAAGG	GCAGAGATGTCAGATGTTGTTC	(GACA)6 (GATA)16
OM-09	GU391028	GGCTACAACACCTGGATGG	TTGGGCTTACTGAAGCTGAC	(GT)26

Supporting Information Table S4: Allele frequencies per population for Southern and Northern *O. korogwe,* and *O. urolepis* from Rufiji river, and *O. placidus* from Lake Chidya.

Locus	Allele	P-C	K-Z	K-M	U-R	K-R	N-R
OMO219	416	0.000	0.083	0.106	0.038	0.406	0.000
OMO219	421	0.000	0.000	0.030	0.173	0.000	0.000
OMO219	426	0.063	0.917	0.864	0.058	0.594	0.857
OMO219	431	0.938	0.000	0.000	0.096	0.000	0.000
OMO219	436	0.000	0.000	0.000	0.250	0.000	0.000
OMO219	441	0.000	0.000	0.000	0.346	0.000	0.000
OMO219	446	0.000	0.000	0.000	0.038	0.000	0.143
OMO229	138	0.000	0.844	1.000	0.000	1.000	0.850
OMO229	141	0.000	0.094	0.000	0.000	0.000	0.000
OMO229	144	0.100	0.063	0.000	0.000	0.000	0.000
OMO229	147	0.000	0.000	0.000	0.100	0.000	0.000
OMO229	150	0.350	0.000	0.000	0.180	0.000	0.000
OMO229	153	0.400	0.000	0.000	0.400	0.000	0.150
OMO229	156	0.100	0.000	0.000	0.120	0.000	0.000
OMO229	159	0.050	0.000	0.000	0.060	0.000	0.000
OMO229	162	0.000	0.000	0.000	0.040	0.000	0.000
OMO229	165	0.000	0.000	0.000	0.080	0.000	0.000
OMO229	174	0.000	0.000	0.000	0.020	0.000	0.000
OMO337	149	0.100	1.000	1.000	0.038	0.941	0.900
OMO337	153	0.750	0.000	0.000	0.327	0.059	0.100
OMO337	157	0.150	0.000	0.000	0.462	0.000	0.000
OMO337	161	0.000	0.000	0.000	0.038	0.000	0.000
OMO391	278	0.000	0.000	0.000	0.000	0.059	0.000
OMO391	281	0.000	0.533	1.000	0.212	0.941	1.000
OMO391	287	0.950	0.233	0.000	0.481	0.000	0.000
OMO391	290	0.050	0.167	0.000	0.000	0.000	0.000
OMO391	293	0.000	0.067	0.000	0.115	0.000	0.000
OMO391	296	0.000	0.000	0.000	0.192	0.000	0.000
OMO392	421	0.000	0.000	0.000	0.000	0.273	0.125
OMO392	426	1.000	0.611	0.775	0.135	0.500	0.625
OMO392	431	0.000	0.333	0.075	0.827	0.227	0.250
OMO392	436	0.000	0.056	0.150	0.000	0.000	0.000
OMO392	441	0.000	0.000	0.000	0.038	0.000	0.000
OMO397	211	0.000	0.000	0.000	0.038	0.000	0.100
OMO397	215	0.000	0.000	0.163	0.058	0.000	0.000
OMO397	219	0.000	0.406	0.000	0.038	0.059	0.000
OMO397	223	0.500	0.000	0.000	0.231	0.000	0.000
OMO397	227	0.056	0.000	0.425	0.327	0.000	0.000
OMO397	231	0.000	0.188	0.413	0.135	0.853	0.900
OMO397	235	0.333	0.406	0.000	0.154	0.088	0.000
OMO397	239	0.111	0.000	0.000	0.019	0.000	0.000
OMO09	194	0.200	0.000	0.000	0.000	0.000	0.000
OMO09	204	0.000	0.813	0.000	0.000	0.813	0.800
OMO09	206	0.000	0.188	0.000	0.000	0.188	0.200
OMO09	212	0.000	0.000	0.000	0.023	0.000	0.000
OMO09	214	0.000	0.000	1.000	0.045	0.000	0.000
OMO09	216	0.000	0.000	0.000	0.045	0.000	0.000
OMO09	218	0.000	0.000	0.000	0.318	0.000	0.000
OMO09	220	0.000	0.000	0.000	0.068	0.000	0.000
OMO09	224	0.300	0.000	0.000	0.023	0.000	0.000
OMO09	226	0.200	0.000	0.000	0.000	0.000	0.000
OMO09	232	0.100	0.000	0.000	0.114	0.000	0.000
OMO09	234	0.200	0.000	0.000	0.045	0.000	0.000
OMO09	236	0.000	0.000	0.000	0.023	0.000	0.000
OMO09	238	0.000	0.000	0.000	0.023	0.000	0.000
OMO09	240	0.000	0.000	0.000	0.045	0.000	0.000

Locus	Allele	P-C	K-Z	K-M	U-R	K-R	N-R
OMO09	244	0.000	0.000	0.000	0.068	0.000	0.000
OMO09	248	0.000	0.000	0.000	0.023	0.000	0.000
OMO09	252	0.000	0.000	0.000	0.023	0.000	0.000
OMO09	254	0.000	0.000	0.000	0.045	0.000	0.000
OMO09	256	0.000	0.000	0.000	0.023	0.000	0.000
OMO09	258	0.000	0.000	0.000	0.023	0.000	0.000
OMO09	260	0.000	0.000	0.000	0.023	0.000	0.000
OMO43	250	0.000	0.000	0.000	0.180	0.000	0.000
OMO43	254	1.000	0.031	0.000	0.080	0.000	0.000
OMO43	258	0.000	0.094	0.000	0.340	0.000	0.000
OMO43	262	0.000	0.875	1.000	0.400	1.000	1.000
OMO129	257	0.000	0.000	0.000	0.846	0.000	0.000
OMO129	261	0.050	0.000	0.000	0.038	0.000	0.000
OMO129	265	0.150	1.000	1.000	0.000	0.471	0.333
OMO129	269	0.050	0.000	0.000	0.096	0.529	0.667
OMO129	273	0.600	0.000	0.000	0.000	0.000	0.000
OMO129	277	0.150	0.000	0.000	0.000	0.000	0.000
OMO129	289	0.000	0.000	0.000	0.019	0.000	0.000
OMO03	164	0.000	0.000	0.000	0.071	0.000	0.000
OMO03	168	0.100	0.200	0.000	0.048	0.000	0.000
OMO03	172	0.000	0.000	0.000	0.048	0.000	0.000
OMO03	176	0.100	0.000	0.000	0.167	0.000	0.000
OMO03	180	0.150	0.000	0.000	0.095	0.188	0.000
OMO03	184	0.000	0.200	0.000	0.024	0.000	0.000
OMO03	188	0.100	0.200	0.000	0.000	0.125	0.500
OMO03	192	0.150	0.000	0.000	0.024	0.188	0.000
OMO03	196	0.000	0.000	0.000	0.167	0.000	0.000
OMO03	200	0.050	0.000	0.000	0.048	0.375	0.500
OMO03	204	0.100	0.000	0.000	0.024	0.125	0.000
OMO03	208	0.050	0.400	0.000	0.024	0.000	0.000
OMO03	212	0.100	0.000	0.000	0.024	0.000	0.000
OMO03	216	0.050	0.000	0.000	0.000	0.000	0.000
OMO03	220	0.050	0.000	0.000	0.000	0.000	0.000
OMO03	252	0.000	0.000	0.000	0.071	0.000	0.000
OMO03	256	0.000	0.000	0.000	0.071	0.000	0.000
OMO03	260	0.000	0.000	0.000	0.095	0.000	0.000
OMO04	177	0.000	0.000	0.000	0.053	0.000	0.000
OMO04	181	0.000	0.000	0.000	0.026	0.000	0.000
OMO04	185	0.000	0.000	0.000	0.079	0.000	0.000
OMO04	189	0.000	0.000	0.000	0.053	0.000	0.000
OMO04	193	0.000	0.000	0.000	0.026	0.000	0.000
OMO04	197	0.000	0.000	0.000	0.053	0.000	0.000
OMO04	201	0.000	0.000	0.100	0.000	0.000	0.000
OMO04	205	0.000	0.000	0.000	0.079	0.000	0.000
OMO04	209	0.000	0.000	0.057	0.000	0.000	0.000
OMO04	213	0.000	0.000	0.000	0.053	0.000	0.000
OMO04	217	0.000	0.000	0.271	0.105	0.000	0.000
OMO04	221	0.000	0.000	0.157	0.053	0.000	0.000
OMO04	225	0.000	0.000	0.271	0.000	0.000	0.000
OMO04	229	0.000	0.000	0.000	0.105	0.000	0.000
OMO04	233	0.000	0.000	0.043	0.000	0.000	0.000
OMO04	237	0.000	0.000	0.029	0.026	0.000	0.000
OMO04	241	0.000	0.000	0.000	0.053	0.000	0.000
OMO04	245	0.000	0.000	0.000	0.079	0.000	0.000
OMO04	249	0.000	0.000	0.000	0.026	0.000	0.000
OMO04	253	0.000	0.000	0.071	0.053	0.000	0.000
OMO04	257	0.000	0.000	0.000	0.053	0.000	0.100
OMO04	261	0.050	0.000	0.000	0.000	0.000	0.000
OMO04	265	0.150	1.000	0.000	0.026	0.029	0.000
OMO04	269	0.050	0.000	0.000	0.000	0.971	0.900

Locus	Allele	P-C	K-Z	K-M	U-R	K-R	N-R
OMO04	273	0.600	0.000	0.000	0.000	0.000	0.000
OMO04	277	0.150	0.000	0.000	0.000	0.000	0.000
OMO01	104	0.0	0.000	0.000	0.023	0.000	0.000
OMO01	106	0.0	0.000	0.086	0.000	0.000	0.000
OMO01	108	0.0	0.036	0.543	0.068	0.000	0.000
OMO01	110	0.0	0.000	0.371	0.023	0.000	0.000
OMO01	112	0.0	0.000	0.000	0.295	0.000	0.000
OMO01	114	0.0	0.000	0.000	0.068	0.000	0.125
OMO01	116	0.0	0.000	0.000	0.045	0.000	0.000
OMO01	118	0.0	0.000	0.000	0.091	0.000	0.000
OMO01	120	0.0	0.000	0.000	0.023	0.000	0.000
OMO01	122	0.0	0.000	0.000	0.068	0.000	0.000
OMO01	124	0.0	0.000	0.000	0.023	0.000	0.000
OMO01	132	0.0	0.000	0.000	0.023	0.000	0.000
OMO01	134	0.0	0.000	0.000	0.023	0.000	0.000
OMO01	136	0.0	0.000	0.000	0.023	0.000	0.000
OMO01	138	0.0	0.000	0.000	0.023	0.000	0.000
OMO01	140	0.0	0.000	0.000	0.045	0.038	0.000
OMO01	142	0.0	0.000	0.000	0.068	0.000	0.000
OMO01	144	0.0	0.000	0.000	0.045	0.000	0.000
OMO01	152	0.0	0.000	0.000	0.000	0.000	0.250
OMO01	156	0.0	0.000	0.000	0.000	0.038	0.000
OMO01	162	0.0	0.679	0.000	0.000	0.538	0.625
OMO01	164	0.0	0.286	0.000	0.000	0.308	0.000
OMO01	168	0.0	0.000	0.000	0.023	0.000	0.000
OMO01	200	0.0	0.000	0.000	0.000	0.077	0.000
OMO114	199	0.0	0.000	0.000	0.240	0.000	0.000
OMO114	205	0.0	0.000	0.163	0.020	0.000	0.000
OMO114	208	0.0	0.000	0.838	0.000	0.462	0.000
OMO114	211	0.0	0.929	0.000	0.360	0.538	0.875
OMO114	214	0.0	0.071	0.000	0.100	0.000	0.000
OMO114	217	0.0	0.000	0.000	0.220	0.000	0.000
OMO114	220	0.0	0.000	0.000	0.040	0.000	0.000
OMO114	223	0.0	0.000	0.000	0.020	0.000	0.000
OMO114	226	0.0	0.000	0.000	0.000	0.000	0.125

References:

Agdamar, S., Tarkan, A.S., Keskin, E., Top, N., Dogac, E., Baysal, O., and Emiroglu, O., 2015. The role of environmental factors and genetic diversity on colonization success of a non-native fish, *Lepomis gibbosus* from western part of Turkey. *Biochemical Systematics and Ecology*, **58**, pp. 195-203.

Agnese, J.F., AdepoGourene, B., Abban, E.K., and Fermon, Y., 1997. Genetic differentiation among natural populations of the Nile Tilapia *Oreochromis niloticus* (*Teleostei, Cichlidae). Genetics and Aquaculture in Africa*, **79**(1), pp. 88-96.

Ahmed, M., and Lorica, M.H. 2002. Improving developing country food security through aquaculture development – lessons from Asia. *Food Policy*, **27**, 125-141.

Alcaraz, C., Vila-Gispert, A., and Gargía-Berthou, E. 2005. Profiling invasive fish species: The importance of phylogeny and human use. *Diversity and Distributions*, **11**(4), 289-298.

Albertson, R.C., and Kocher, T.D., 2005. Genetic architecture sets limits on transgressive segregation in hybrid cichlid fishes. *Evolution*, **59**(3), pp. 686-690.

Allan, J.D., Abell, R., Hogan, Z., Revenga, C., Taylor, B.W., Welcomme, R.L., and Winemiller, K., 2005. Overfishing of inland waters. *Bioscience*, **55**(12), pp. 1041-1051.

Allendorf, F.W., Leary, R.F., Spruell, P., and Wenburg, J.K., 2001. The problems with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution*, **16**, 613-622.

Ambali, A.J.D., Doyle, R.W., and Cook, D.I., 1999. Genetic changes in *Oreochromis shiranus* (Trewavas) associated with the early stages of national aquaculture development in Malawi. *Aquaculture Research*, **30**, 579-588.

Angienda, P.O., Lee, H.J., Elmer, K.R., Abila, R., Waindi, E.N., and Meyer, A., 2011. Genetic structure and gene flow in an endangered native fish (*Oreochromis esculentus)* compared to invasive Nile Tilapia (*Oreochromis niloticus*) in Yala swamp, East Africa. *Conservation Genetics*, **12**, 243-255.

Arnold, M.L., and Hodges, S.A., 1995. Are natural hybrids fit or unfit relative to their parents? *Tree*, **10**, 67-71.

Arnold, M.L., and Martin, N.H., 2010. Hybrid fitness across time and habitats. *Trends in Ecology and Evolution*, **25**, 503-536.

Arroita, M., Flores, L., Larrañaga, A., Martínez, A., Martínez-Santos, M., Pereda, O., Ruiz-Romera, E., Solagaistua, L., and Elosegi, A. 2017. Water abstraction impacts stream ecosystem functioning via a wetted-channel contraction. *Freshwater Biology*, 62, pp. 243-257.

Barasa, J.E., Abila, J.P., Dangasuk, O.G., Njahira, M.N., and Kaunda-Arara, B., 2014. Genetic diversity and gene flow in *Clarius gariepinus* from Lakes Victoria and Kanyaboli Kenya. *African Journal of Aquatic Science*, **39**, 287-293.

Barel., C.D.N., van Oijen, M.J.P., Witte, F., and Witte-Maas, E.L.M., 1977. An introduction to the taxonomy and morphology of the Haplochromine *Cichlidae* from Lake Victoria. *Netherlands Journal of Zoology*, **27**, 381-389.

Bartley, D.M., Rana, K., and Immink, A.J., 2001. The use of inter-specific hybrids in aquaculture and fisheries. *Reviews in Fish Biology and Fisheries*, **10**, pp. 325-337.

Bartley, D.M., De Graaf, G.J., Valbo-Jørgensen, J., and Marmulla, G., 2015. Inland capture fisheries: status and data issues. *Fisheries Management Ecology,* **22**: 71–77.

Barton, N.H., 2001. The role of hybridization in evolution. *Molecular Ecology*, **10**, pp. 551-568.

Becher, C., and Gumm, J.M., 2018. The roles of inter-and intra-sexual selection in behavioural isolation between native and invasive pupfishes. *Current Zoology*, **64**, 135-144.

Belk, M.C., and Schaalje, G.B., 2016. Multivariate heritability of shape in June sucker (*Chasmistes liorus*) and Utah sucker (*Catostomus ardens*): shape as a functional trait for discriminating closely related species. *Developmental Genetics and Evolution*, **226**, 197-207.

Belkhir K., Borsa P., Chikhi L., Raufaste N. and Bonhomme F., 1999. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier (France).

Bentsen, H.B., Gjerde, B., Eknath, A.E., de Vera, M.S.P., Velasco, R.R., Danting, J.C., Dionisio, E.E., Longalong, F.M., Reyes, R.A., Abella, T.A., Tayamen, M.M., and Ponzoni, R.W., 2017. Genetic improvement of farmed tilapias: Response to five generations of selection for increased body weight at harvest in *Oreochromis niloticus* and the further impact of the project. *Aquaculture,* **468**, 206-217.

Bernatchez, L., Wellenreuther, M., Araneda, C., Ashton, D.T., Barth, J.M.I., Beacham, T.D., Maes, G.E., Martinsohn, J.T., Miller, K.M., Naish, K.A., Ovenden, J.R., Primmer, C.R., Suk, H.Y., Therkildsen, N.O., and Withler, R.E., 2017. Harnessing the power of genomics to secure the future of seafood. *Trends in Ecology and Evolution*, **32**, 665-680.

Bezault, E., Balaresque, P., TOguyeni, A., Fermon, Y., Araki, H., Baroiller, J.F., and Rognon, X., 2011. Spatial and temporal variation in population genetic structure of wild Nile tilapia (*Oreochromis niloticus*) across Africa. *BMC Genetics*, **12**, DOI: 10.1186/1471-2156-12-102.

Blackburn, T.M., Pysek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarosik, V., Wilson, J.R.U., and Richardson, D.M., 2011. A proposed unified framework for biological invasions. *Trends in Ecology and Evolution*, **26**, 333-339.

Boecklen, W.J. and Howard, D.J., 1997. Genetic analysis of hybrid zones: numbers of markers and power of resolution. *Ecology*, **78**, 2611–2616.

Bradbeer, S.J., Harrington, J., Watson, H., Warraich, A., Shechonge, A., Smith, A., Tamatamah, R., Ngatunga, B.P., Turner, G.F., and Genner, M.J., 2018. Limited hybridization between introduced and critically endangered indigenous tilapia fishes in northern Tanzania. *Hydrobiologia*, Available at: https://doi.org/10.1007/s10750-018-3572-5 [Accessed June 2018].

Britton, A.W., Day, J.J., Doble, C.J., Ngatunga, B.P., Kemp, K.M., Carbone, C., and Murrell, D.J., 2017. Terrestrial-focused protected areas are effective for conservation of freshwater fish diversity in Lake Tanganyika. *Biological Conservation*, **212**, 120-129.

Brooks, E.G.E., Holland, R.A., Darwall, W.R.T., and Eigenbrod, F. 2016. Global evidence of positive impacts of freshwater biodiversity on fishery yields. *Global Ecology and Biogeography*, **25**, 553-562.

Brooks, J.L., Boston, C., Doka, S., Gorsky, D., Gustavson, K., Hondorp, D., Isermann, D., Midwood, J.D., Pratt, T.C., Rous, A.M., Withers, J.L., Krueger, C.C., and Cooke, S.J., 2017. Use of fish telemetry in rehabilitation planning, management and monitoring in areas of concern in the Laurentian Great Lakes. *Environmental Management*, **60**, 1139-1154.

Brummer, E.C., Barber, W.T., Collier, S.M., Cox, T.S., Johnson, R., Murray, S.C., Olsen, R.T., Pratt, R.C., and Thro, A.M. 2011. Plant breeding for harmony between agriculture and the environment. *Frontiers in Ecology and the Environment,* **9**, 561–568.

Brummett, R.E., and Ponzoni, R.W., 2009. Improved strains of Tilapia in African aquaculture. *Reviews in Fisheries Science*, **17**, 70-77.

Brummett, R.E., and Williams, M.J., 2000. The evolution of aquaculture in African rural and economic development. *Ecological Economics*, **33**, 193-203.

Burress, E.D., 2015. Cichlid fishes as models of ecological diversification: patterns, mechanisms, and consequences. *Hydrobiologica*, **748,** 7-27.

Burress, E.D., 2016. Ecological diversification associated with the pharyngeal jaw diversity of Neotropical cichlid fishes. *Journal of Animal Ecology*, **85**, 302-313.

Bush, A., Hermoso, V., Linke, S., Nipperess, D., Turak, E., and Hughes, L., 2014. Freshwater conservation planning under climate change: demonstrating proactive approaches for Australian Odonata. *Journal of Applied Ecology*, **51**, 1273-1281.

Bush, A., and Hoskins, A.J., 2017. Does dispersal capacity matter for freshwater biodiversity under climate change? *Freshwater Biology*, **62**, 382-396.

Canonico, G.C., Arthington, A., McCrary, J.K. and Thieme, M.L., 2005. The effects of introduced tilapias on native biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems* **15**, 463-483.

Chao, B.F., 1995. Anthropogenic impact on global geodynamics due to reservoir water impoundment. *Geophysical Research Letters*, **22**, 3529–3532.

Chappell, B.F., and Smith, K.G., 2016. Patterns of predation of native reef fish by invasive Indo-Pacific lionfish in the western Atlantic: Evidence of selectivity by a generalist predator. *Global Ecology and Conservation*, **8**, 18-23.

Cohen, A.S., Gergurich, E.L., Kraemer, B.M., McGlue, M.M., McIntyre, P.B., Russell, J.M., Simmons, J.D., and Swarzenski, P.W., 2016. Climate warming reduces fish production and benthic

habitat in Lake Tanganyika, one of the most biodiverse freshwater ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 9563-9568.

Colas, F., Baudoin, J., Chauvet, E., Clivot, H., Danger, M., Guerold, F., and Devin, S., 2016. Dam-associated multiple-stressor impacts on fungal biomass and richness reveal the initial signs of ecosystem functioning impairment. *Ecological Indicators*, **60**, 1077-1090.

Coleman, R.R., Gaither, M.R., Kimokeo, B., Stanton, F.G., Bowen, B.W., and Toonen, R.J., 2014. Large-scale introduction of the Indo-Pacific damselfish *Abudefduf vaigiensis* into Hawai'i promotes genetic swamping of the endemic congener *A. abdominalis*. *Molecular Ecology*, **23**, 5552-5565.

Colombo, M., Indermaur, A., Meyer, B.S., and Salzburger, W. 2016. Habitat use and its implication to functional morphology: niche partitioning and the evolution of locomotory morphology in Lake Tanganyikan cichlids (Perciformes: Cichlidae). *Biological Journal of the Linnean Society*, **117**, pp. 536-550.

Conte, M.A., Gammerdinger, W.J., Bartie, K.L., Penman, D.J., and Kocher, T.D., 2017. A high quality assembly of the Nile Tilapia (*Oreochromis niloticus*) genome reveals the structure of two sex determination regions. *BMC Genomics*, **18**.

Costanza R., d'Arge R., de Groot R., Farber S., Grasso M., Hannon B., Limberg, K., Naeem, S., ONeill, R.V., Pruelo, J., Raskin, R.G., Sutton, P., and vandenBelt, M., 1997. The value of the world's ecosystem services and natural capital. *Nature*, **387**, 253–260.

Costedoat, C., Perch, N., Salducci, M., Chappaz, R., and Gilles, A., 2005. Evolution of mosaic hybrid zone between invasive and endemic species of *Cyprinidae* through space and time. *Biological Journal of the Linnean Society*, **85**, 135-155.

D'Amato, M.E., Esterhuyse, M.M., Van Der Waal, B.C., Brink, D. and Volckaert, F.A., 2007. Hybridization and phylogeography of the Mozambique tilapia *Oreochromis mossambicus* in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. *Conservation Genetics*, ***8***, 475-488.

Darwall, W., Smith, K., Lowe, T. and Vié, J.C., 2005. *The status and distribution of freshwater biodiversity in Eastern Africa*. IUCN SSC Freshwater Biodiversity Assessment Programme. IUCN, Gland, Switzerland and Cambridge, UK.

Deines, A.M., Bbole, I., Katongo, C., Feder, J.L. and Lodge, D.M., 2014. Hybridization between native *Oreochromis* species and introduced Nile tilapia *O. niloticus* in the Kafue River, Zambia. *African Journal of Aquatic Science*, **39**, 23-34.

Deines, A.M., Bunnell, D.B., Rogers, M.W., Bennion, D., Woelmer, W., Sayers, M.J., Grimm, A.G., Shuchman, R.A., Raymer, Z.B., Brooks, C.N., Mychek-Londer, J.G., Taylor, W., and Beard Jr., T.D., 2017. The contribution of lakes to global inland fisheries harvest. *Frontiers in Ecology and the Environment*, **15**, 293-298.

Dieleman, J., Van Bocxlaer, B., Manntschke, C., Nyingi, D.W., Adriaens, D. and Verschuren, D., 2015. Tracing functional adaptation in African cichlid fishes through morphometric analysis of fossil teeth: exploring the methods. *Hydrobiologia*, **755**, 73-88.

Dieleman, J., Muschick, M., Nyingi, W.D., and Verschuren, D., 2018. Species integrity and origin of *Oreochromis hunteri* (*Pisces: Cichlidae*), endemic to crater Lake Chala (Kenya-Tanzania). *Hydrobiologia,* available at: https://doi.org/10.1007/s10750-018-3570-7 [Accessed June 2018].

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z., Knowler, D.J., Leveque, C., Naiman, R.J., Prieur-Richard, A., Soto, D., Stiassny, M.L.J., and Sullican, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, **81**, 163-182.

Dunz, A.R., and Schliewen, U.K. 2013. Molecular phylogeny and revised classification of the haplotilapiine cichlid fishes formerly referred to as "Tilapia". *Molecular Phylogenetics and Evolution*, **68**, 64-80.

Eknath, A.E., and Hulata, G., 2009. Use and exchange of genetic resources of Nile Tilapia (*Oreochromis niloticus*). *Reviews in Aquaculture*, **1**, 197-213.

Ellender, B.R., Weyl, O.L.F., and Winker, H., 2012. Age and growth and maturity of southern Africa's largest cyprinid fish, the largemouth yellowfish *Labeobarbus kimberleyensis*. *Journal of Fish Biology*, **81**, 1271–1284, available at: http://dx.doi.org/10.1111/j.1095-8649.2012.03395.x [Accessed January 2018].

Ellender, B.R., and Weyl, O.L.F., 2014. A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. *Aquatic Invasions*, **9**, 117-132.

Ellender, B.R., Woodford, D.J., Weyl, O.L.F., and Cowx, I.G., 2014. Managing conflicts arising from fisheries enhancements based on non-native fishes in southern Africa. *Journal of Fish Biology*, **85**, 1890-1906.

Ellstrand, N.C., and Schierenbeck, K.A., 2006. Hybridization as a stimulus for the evolution of invasiveness in plants?*. *Euphytica*, **148**, 35-46.

Evanno, G., Regnaut, S., and Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611-2620.

Excoffier, L. and Lischer, H.E.L., 2010. Arlequin suite version 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564-567.

FAO, 2016. *The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all.* FAO, Rome.

Feurtey, A., Cornille, A., Shykoff, J.A., Snirc, A., and Giraud, T., 2017. Crop-to-wild gene flow and its fitness consequences for a wild fruit tree: Towards a comprehensive conservation strategy of the wild apple in Europe. *Evolutionary Applications*, **10**, 180-188.

Firmat, C., Alibert, P., Losseau, M., Baroiller, J.F. and Schliewen, U.K., 2013. Successive invasion-mediated interspecific hybridizations and population structure in the endangered cichlid *Oreochromis mossambicus*. *PloS One*, **8**, e63880.

Froese, R., and Pauly. D., Editors. 2018. *FishBase*. World Wide Web electronic publication. Available at: www.fishbase.org, version (02/2018). [Accessed May 2018].

Gainsford, A., 2014. Hierarchical behavior, habitat use, and species size differences shape evolutionary outcomes of hybridization in a coral reef fish. *Journal of Evolutionary Biology*, **28**, 205-222.

Garcia, S.M., Kolding, J., Rice, J., Rochet, M.J., Zhou, S., Arimoto, T., Beyer, J.E., Borges, L., Bundy, A., Dunn, D., Fulton, E.A., Hall, M., Heino, M., Law, R., Makino, M., Rijnsdorp, A.D., Simard, F., and Smith, A.D.M., 2012. Reconsidering the consequences of selective fisheries. *Science, New Series*, **335**, 1045-1047.

Gay, L., Crochet, P.A., Bell, D.A., and Lenormand, T., 2008. Comparing clines on molecular and phenotypic traits in hybrid zones: A window on tension zone models. *Evolution*, **62**, 2789–2806.

Genner, M.J., Nichols, P., Carvalho, G.R., Robinson, R.L., Shaw, P.W., and Turner, G.F., 2007. Reproductive isolation among deep-water cichlid fishes of Lake Malawi differing in monochromatic male breeding dress. *Molecular Ecology*, **16**, 651-662.

Gil. J., Labonne, J., Caudron, A., 2016. Evaluation of strategies to conserve and restore intraspecific biodiversity of brown trout: outcomes from genetic monitoring in the French Alps. *Reviews in Fish Biology and Fisheries,* **26**, 1-11.

Gilbert, L.E., 2003. Adaptive novelty through introgression in Heliconius wing patterns: evidence for shared genetic "tool box" from synthetic hybrid zones and a theory of diversification. In Ecology and Evolution Taking Flight: Butterflies as Model System (Boggs, C.L. et al., eds), 281–318, University of Chicago Press.

Grant, P.R., and Grant, B.R., 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science, New Series*, **296**, 707-711.

Grant, P.R., Grant, B.R., Keller, L.F., Markert, J.A., and Petren, K., 2003. Inbreeding and interbreeding in Darwin's finches. *Evolution*, **57**, 2911-2916.

Grant, W.S., 2012. Understanding the adaptive consequences of hatchery-wild interactions in Alaska salmon. *Environmental Biology of Fishes*, **94**, 325-342.

Guyon, R., Rakotomanga, M., Azzouzi, N., Coutanceau, J.P., Bonillo, C., D'Cotta, H., Pepey, E., Soler, L., Rodier-Goud, M., D'Hont, A., Conte, M.A., van Bers, N.E.M., Penman, D.J., Hitte, C., Crooijmans, R.P.M.A., Kocher, T.D., Ozouf-Costaz, C., Baroiller, J.F., and Galibert, F., 2012. A high-resolution map of the Nile tilapia genome: a resource for studying cichlids and other percomorphs. *BMC Genomics,* **13**, 1-17.

Hahn, C., Genner, M.J., Turner, G.F., and Joyce, D.A. 2017. The genomic basis of adaptation to the deep water 'twilight zone' in Lake Malawi cichlid fishes. bioRxiv, 102830; DOI: https://doi.org/10.1101/102830.

Hallerman, E., and Hilsdorf, A.W.S., 2014. Conservation genetics of tilapias: Seeking to define appropriate units for management. *Israeli Journal of Aquaculture*, **66**.

Hardig, T.M., Brunsfeld, S.J., Fritz, R.S., Morgan, M., and Orian, C.M., 2000. Morphological and molecular evidence for hybridization and introgression in a willow (*Salix*) hybrid zone. *Molecular Ecology*, **9**, 9-24.

Harrison, R.G., 1990. Hybrid zones: windows on evolutionary process. *Oxford Surveys in Evolutionary Biology,* **7**, 69–128.

Hasselman, D.J., Argo, E.E., Mcbride, M.C., Bentzen, P., Schultz, T.F., Perez-Umphrey, A.A., and Palkovacs, E.P., 2014. Human disturbance causes the formation of a hybrid swarm between two naturally sympatric fish species. *Molecular Ecology*, **23**, 1137-1152.

Hayden, B., Pulcini, D., Kelly-Quinn, M., O'Grady, M., Caffrey, J., McGrath, A., and Mariani, S., 2010. Hybridization between two cyprinid fishes in a novel habitat: genetics, morphology and life-history traits. *BMC Evolutionary Biology*, **10**, 1-11.

Hayden, B., Massa-Gallucci, A., Caffrey, J., Harrod, C., Mariani, S., O'Grady, M., and Kelly-Quinn, M., 2011. Trophic dynamics within a hybrid zone – interactions between an abundant cyprinid hybrid and sympatric parental species. *Freshwater Biology*, **56**, 1723-1735.

Henriques, R., von der Heyden, S., Matthee, C.A., 2016. When homoplasy mimics hybridization: a case study of Cape hakes (*Merluccius capensis* and *M-paradoxus*). *Peer Journals*, **4**.

Hermoso, V., Clavero, M., Blanco-Garrido, F., and Prenda, J., 2011. Invasive species and habitat degradation in Iberian streams: an analysis of their role in freshwater fish diversity loss. *Ecological Applications*, **21**, 175-188.

Hishamunda, N., and Ridler, N.B, 2006. Farminf fish for profits: A small step towards food security in sub-Saharan Africa. *Food Policy*, **31**, 401-414.

Horreo, J.L., Ayllon, F., Perez, J., Beall, E., Garcia-Vazquez, E., 2011. Interspecific hybridization, a matter of pioneering? Insights from Atlantic salmon and Brown trout. *Journal of Heredity*, **102**, 237-242.

Hubbs, C.L., 1955. Hybridization between fish species in nature. *Systematic Zoology*, **4**, 1-20.

Hubisz, H.M.J., Falush, D., Stephens, M., and Pritchard, J.K., 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Research,* **9,** 1322-1332.

Huxel, G.R., 1999. Rapid displacement of native species by invasive species: effects of hybridization. *Biological conservation*, **89**, 143-152.

Januchowski-Hartley, S.R., Holtz, L.A., Martinuzzi, S., McIntyre, P.B., Radeloff, V.C., and Pracheil, B.M., 2016. Future land use threats to range-restricted fish species in the United States. *Diversity and Distributions,* **22**, 663-671.

Jarne, P., and Lagoda, P.J.L., 1996. Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, **11,** 424-429.

Kagawa, K., and Takimoto, G., 2018. Hybridization can promote adaptive radiation by means of transgressive segregation. *Ecology Letters*, **21**, 264-274.

Kalacska, M., Arroyo-Mora, J.P., Lucanus, O., and Kishe-Machumu, M.A., 2017. Land cover, land use, and climate change impacts on endemic cichlid habitats in northern Tanzania. *Remote Sensing*, **9**, 623.

Kano, Y., Dudgeon, D., Nam, S., Samejima, H., Watanabe, K., Grudpan, C., Grudpan, J., Magtoon, W., Musikasinthorn, P., Nguyen, P.T., Praxaysonbath, B., Sato, T., Shibukawa, K., Shimatani, Y., Suvarnaraksha, A., Tanaka, W., Thach, P., Tran, D.D., Yamashita, T., and Utsugi, K., 2016. Impacts of dams and global warming on fish biodiversity in the Indo-Burma Hotspot. *PLoS One*, **11**, e0160151.

Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, **11**, 353-357.

Koblmuller, S., Duftner, N., Sefc, K.M., Aibara, M., Stipacek, M., Blanc, M., Egger, B., and Sturmbauer, C., 2007. Reticulate phylogeny of gastropod-shell-breeding cichlids from Lake Tanganyika – the result of repeated introgressive hybridization. *BMC Evolutionary Biology*, **7**.

Kolding, J., and van Zwieten, P.A.M., 2012. Relative lake level fluctuations and their influence on productivity and resilience in tropical lakes and reservoirs. *Fisheries Research*, **1115-116**, 99-109.

Koolboon, U., Koonawootrittriron, S., Kamolrat, W., and Na-Nakorn, U., 2014. Effects of parental strains and heterosis of the hybrid between *Clarias macrocephalus* and *Clarias gariepinus*. *Aquaculture*, **424**, 131-139.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A. and Mayrose, I., 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, **15**, 1179-1191.

Lamb, T., and Avise, J.C., 1987. Morphological variability in genetically defined categories of anuran hybrids. *Evolution*, **41**, 157-165.

Lehosmaa, K., Jyvasjarvi, J., Virtanen, R., Ilmonen, J., Saastamoinen, J., Muotka T., 2017. Anthropogenic habitat disturbance induces a major biodiversity change in habitat specialist bryophytes of boreal springs. *Biological Conservation*, **215**, 169-178.

Lepori, F., Benjamin, J.R., Fausch, K.D., and Baxter, C.V., 2012. Are invasive and native trout functionally equivalent predators? Results and lessons from a field experiment. *Aquatic Conservation – Marine and Freshwater Ecosystems*, **22**, 787-798.

Levin, D.A., Francisco-Ortega, J., and Jansen, R.K., 1996. Hybridization and the extinction of rare plant species. *Conservation Biology*, **10**, 10-16.

Lind, C.E., Brummett, R.E. and Ponzoni, R.W., 2012a. Exploitation and conservation of fish genetic resources in Africa: issues and priorities for aquaculture development and research. *Reviews in Aquaculture*, **4**, 125-141.

Lind. C.E., Ponzoni, R.W., Nguyen, N.H., and Khaw, H.L., 2012b. Selective breeding in fish and conservation genetic resources for aquaculture. *Reproduction in Domestic Animals*, **47**, 255-263.

Liu, F., Sun, F., Li, J., Xia, J.H., Lin, G., Tu, R.J., and Yue, G.H., 2013. A microsatellite-based linkage map of salt tolerant tilapia (*Oreochromis mossambicus* x *Oreochromis spp*.) and mapping of sex-determining loci. *BMC Genomics*, **14**, 1-14.

Lowe, R.H., 1955. New species of *Tilapia* (Cichlidæ) from Lake Jipe and the Pangani River, East Africa, with notes on the biology of these and Lake Victoria species grown in ponds. *Bulletin of the British Museum of Natural History*, **2**, 350–368.

Lowe-McConnell, R.H., 2006. *The tilapia trail – the life story of a fish biologist*. Ascot: MPM Publishing.

Lynch, A.J., Cowx, I.G., Fluet-Chouinard, E., Glaser, S.M., Phang, S.C., Beard, T.D., Bower, S.D., Brooks, J.L., Bunnell, D.B., Claussen, J.E., Cooke, S.J., Kao, Y.C., Lorenzen, K., Myers, B.J.E., Reid, A.J., Taylor, J.J., and Youn, S., 2017. Inland fisheries - Invisible but integral to the UN Sustainable Development Agenda for ending poverty by 2030. *Global Environmental Change-Human and Policy Dimensions*, **47**, 167-173.

Maan, M.E., Seehausen, O., and Groothuis, T.G.G., 2017. Differential survival between visual environments supports a role of divergent sensory drive in cichlid fish speciation. *The American Naturalist*, **189,** 78-85.

Maitipe, P., and DeSilva, S.S., 1985. Switches between zoophagy, phytophagy and detritivory of *Sarotherodon mossambicus* (Peters) Populations in 12 man-made Sri-Lankan lakes. *Journal of Fish Biolgoy*, **26**, 49-61.

Malinsky, M., Challis, R.J., Tyers, A.M., Schiffels, S., Terai, Y., Ngatunga, B.P., Miska, E.A., Durbin, R., Genner, M.J., and Turner, G.F., 2015. Genomic islands of speciation separate cichlid ectomorphs in an East African crater lake. *Science*, **350**, 1493-1498.

Mallet, J., 2005. A species definition for the modern synthesis. *Trends in Ecology and Evolution*, **10**, 294-299.

Maluwa, A.O., and Gjerde, B., 2007. Response to selection for harvest body weight of *Oreochromis shiranus. Aquaculture*, **273**, 33–41.

Mantel, S.K., Rivers-Moore, N., and Ramulifho, P., 2017. Small dams need consideration in riverscape conservation assessments. *Aquatic Conservation – Marine and Freshwater Ecosystems*, **27**, 48-754.

Marr, S.M., Marchetti, M.P., Olden, J.D., Garcia-Berthou, E., Morgan, D.L., Arismendi, I., Day, J.A., Griffiths, C.L., and Skelton, P.H., 2010. Freshwater fish introductions in Mediterranean-climate regions: are there commonalities in the conservation problem? *Diversity and Distributions*, **16**, 606-619.

Martinuzzi, S., Januchowski-Hartley, S.R., Pracheil, B., McIntyre, P.B., Plantinga, A.J., Lewis, D.J., and Radeloff, V.C., 2014. Threats and opportunities for freshwater conservation under future land use change scenarios in the United States. *Global Change Biology*, **20**, 113-124.

McAndrew, B.J., Roubal, F.R., Roberts, R.J., Bullock, A.M., and McEwen, I.M., 1988. The genetics and histology of red, blond, and associated colour variants in *Oreochromis niloticus. Genetica*, **76**, 127-137.

McCafferty, J.R., Ellender, B.R.,Weyl, O.L.F.and Britz, P.J., 2012. The use of water resources for inland fisheries in South Africa. *Water SA,* **38,** 327–344.

McIntyre, P.B., Michel, E., France, K., Rivers, A., Hakizimana, P., and Cohen, A.S., 2005. Individual- and assemblage-level effects of anthropogenic sedimentation on snails in Lake Tanganyika. *Conservation Biology*, **19**, 171-181.

McIntyre, P.B., Liermann, C.A.R., and Revenga, C., 2016. Linking freshwater fishery management to global food security and biodiversity conservation. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 12880-12885.

Meyer J, McDowell W, Bott, T.L., Elwood, J.W., Ishizaki, C., Melack, J.M., Peckarsky, B.L., Peterson, B.J., and Rublee, P.A., 1988. Elemental dynamics in streams. *Journal of the North American Benthological Society*, **7**, 410–432.

Millennium Ecosystem Assessment, 2016. 'Ecosystems and human well-being'. *Washington, DC*.

Montanari, S.R., Hobbs, J.P.A., Pratchett, M.S., and van Herwerden, L., 2016. The importance of ecological and behavioural data in studies of hybridization among marine fishes. Reviews in Fish Biology and Fisheries, 26, 181-198.

Moralee, R.D., van der Bank, F.H., and van der Waal, B.C.W., 2000. Biochemical genetic markers to identify hybrids between the endemic Oreochromis mossambicus and the alien species, *O. niloticus* (*Pisces : Cichlidae*). *Water SA*, **26**, 263-268.

Morgado-Santos, M., Pereira, H.M., Vicente, L., and Collares-Pereira, M.J., 2015. Mate choice drives evolutionary stability in a hybrid complex. *PLoS One*, **19**, e0132760.

Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F., and Allendorf, F.W. 2018. Hybridization rapidly reduces fitness of a native trout in the wild. *Biology Letters,* DOI: 10.1098/rsbl.2009.0033.

Na-Nakorn, U., and Brummett, R.E., 2009. Use and exchange of aquatic genetic resources for food and aquaculture: *Clarius* catfish. *Reviews in Aquaculture*, **1**, 214-223.

Napit, M.K., 2013. The effect of pesticides on fish fauna of Bhopal lower lake (M.P.). *African Journal of Environmental Science*, **7**, 745-727.

Ndiwa, T.C., Nyingi, D.W. and Agnese, J.F., 2014. An important natural genetic resource of *Oreochromis niloticus* (Linnaeus, 1758) threatened by aquaculture activities in Loboi drainage, Kenya. *PloS One*, **9**, e106972.

Neeson, T.M., Ferris, M.C., Diebel, M.W., Doran, P.J., O'Hanley, J.R., and McIntyre, P.B., 2015. Enhancing ecosystem restoration efficiency through spatial and temporal coordination. *Proceedings of National Academy of Science*, **112**, 6236-6241.

Nilsson C., Reidy C.A., Dynesius, M., and Revenga, C., 2005 Fragmentation and flow regulation of the world's large river systems. *Science*, **308**, 405–408.

Nobah, C.S.K., Kone, T., Ouattara, I.N., Kouamelan, P.E., N'Douba, V., Snoeks, J., 2008. Study of the growth performances of two tilapias (*Tilapia zillii* and *T-guineensis*) and their hybrids in a floating cage. *Cybium,* **32**, 131-136.

Nyingi, D.W. and Agnèse, J.F., 2007. Recent introgressive hybridization revealed by exclusive mtDNA transfer from *Oreochromis leucostictus* (Trewavas, 1933) to *Oreochromis niloticus* (Linnaeus, 1758) in Lake Baringo, Kenya. *Journal of Fish Biology*, **70**, 148-154.

O'Connell, M., and Wright, J.M., 1997. Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries*, **7**, 331-363.

Ogutu-Ohwayo, R., 1990. The decline of the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus*. *Environmental Biology of Fishes*, **27**, 81-96.

Okomoda, V.T., Koh, I.C.C., and Shahreza, M.S., 2017. First report on the successful hybridization of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822). *Zygote*, **25**, 443-452.

Olden, J.D., and Naiman, R.J., 2010. Incorporating thermal regimes into environmental flows assessments: modifying dam operations to restore freshwater ecosystem integrity. *Freshwater Biology*, **55**, 86–107.

Olden, J.D., Kennard, M.J., Leprieur, F., Tedesco, P.A., Winemiller, K.O., Garcia-Berthou, E., 2010. Conservation biogeography of freshwater fishes: recent progress and future challenges. *Diversity and Distributions*, **16**, 496-513.

Oso, J.A., Ayodele, I.A., and Fagbuaro, O., 2006. Food and feeding habits of *Oreochromis niloticus* (L.) and *Sarotherodon galilaeus* (L.) in a Tropical Reservoir. *World Journal of Zoology*, **1**, 118-121.

Parsons, K.J., Son, Y.H., and Albertson, R.C., 2011. Hybridization promotes evolvability in African cichlids: Connections between transgressive segregation and phenotypic integration. *Evolutionary Biology*, **38**, 306-315.

Parveen, S., Abbas, K., Afzal, M., and Hussain, M., 2018. Prediction of potential hybridization between three major carps in Ravi river (Punjab, Pakistan) basin by using microsatellite markers. *Turkish Journal of Fisheries and Aquatic Sciences*, **18**, 27-35.

Pearce, D., 1998. Auditing the Earth: the value of the worlds ecosystem services and natural capital. *Environment*, **40**, 23–27.

Piria, M., Simonović, P., Kalogianni, E., Vardakas, L., Koutsikos, N., Zanella, D., Ristovska, M., Apostolou, A., Adrović, A., Mrdak, D., Tarkan, A.S., Milošević, D., Zanella, L.N., Bakiu, R., Ekmekçi, F.G., Povž, M., Korro, K., Nikolić, V., Škrijelj, R., Kostov, V., Gergori, A., and Joy, M.K., 2018. Alien freshwater fish species in the Balkans-Vectors and pathways of introduction. *Fish and Fisheries*, **19**, 138-169.

Poff, N.L., Allan, J.D., Palmer, M.A., Hart, D.D., Richter, B.D., Arthington, A.H., Rogers, K.H., Meyers, J.L., Stanford, J.A., 2003. River flows and water wars: Emerging science for environmental decision making. *Frontiers in Ecology and the Environment*, **1**, 298-306.

Pouyaud, L., and Agnese, J.F., 1995. Phylogenetic relationships between 21 species of three *Tilapine* genera *Tilapia, Sarotherodon* and *Oreochromis* using allozyme data. *Journal of Fish Biology*, **47**, 26-38.

Pritchard, J.K., Stephens, M., and Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945-959.

Pukk, L., Gross, R., Vetemaa, M., Vasemagi, A., 2016. Genetic discrimination of brackish and freshwater populations of Eurasian perch (*Perca fluviatilis L.*) in the Baltic Sea drainage: Implications for fish forensics. *Fisheries Research*, **183**, 155-164.

R Development Core Team (2008) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org.

Rahel, FJ. 2007. Biogeographic barriers, connectivity and homogenization of freshwater faunas: it's a small world after all. *Freshwater Biology*, **52**, 696-710.

Rader, R.B., Belk, M.C., Shiozawa, D.K., and Crandall, K.A., 2005. Empirical test for ecological exchangeability. *Animal Conservation*, **8**, 239–247.

Rieseberg, L. H., Kim, M.J., and Seiler, G.J., 1999a. Introgression between cultivated sunflowers and a sympatric wild relative, *Helianthus petiolaris* (*Asteraceae*). *International Journal of Plant Sciences*, **160**, 102-108.

Rieseberg, L. H., J. Whitton, and K. Gardner. 1999b. Hybrid zones and the genetic architecture of a barrier to gene flow between two wild sunflower species. *Genetics*, **152**, 713-727.

Rhymer, J.M., and Simberloff, D., 1996. Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics*, **27**, 83-109.

Richards, Z.T., and Hobbs, J.P.A., 2015. Hybridization on coral reefs and the conservation of evolutionary novelty. *Current Zoology,* **61**, 132-145.

Roberts, D.G., Gray, C.A., West, R.J., and Ayre, D.J., 2009. Evolutionary impacts of hybridization and interspecific gene flow on an obligately estuarine fish. *Journal of Evolutionary Biology*, **22**, 27-35.

Roberts, D.G., Gray, C.A., West, R.J., and Ayre, D.J., 2010. Marine genetic swamping: Hybrids replace an obligately estuarine fish. *Molecular Ecology*, **19**, 508-520.

Rognon, X., and Guyomard, R., 2003. Large extent of mitochondrial DNA transfer from *Oreochromis aureus* to *O. niloticus* in West Africa. *Molecular Ecology*, **12**, 435-445.

Rohlf, F.J., 2004. TpsDig Version 1.4. Department of Ecology and Evolution. State University of New York at Stony Brook, *New York*.

Rousset F., 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources,* **8**, 103-106.

Ruesink, J.L., 2005. Global analysis of factors affecting the outcome of freshwater fish introductions. *Conservation Biology*, **19**, 1883-1893.

Saju, J.M., Lee, W-J., and Orban, L. 2010. Characterization of nine novel microsatellites isolated from Mozambique tilapia, *Oreochromis mossambicus*. *Conservation Genetics Resources*, **2**, 287–385.

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, R., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., and Wall, D.H., 2000. Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770-1774.

Sales, N.G., Pessali, T.C., Neto, F.R.A., and Carvalho, D.C., 2018. Introgression from non-native species unveils a hidden threat to the migratory Neotropical fish *Prochilodus hartii. Biological Invasions*, **20**, 555-566.

Salzburger, W., Van Bocxlaer, B., and Cohen, A.S., 2014. Ecology and evolution of the African Great Lakes and their faunas. *Annual Review of Ecology, Evolution, and Systematics*, **45**, 519-522.

Saunders, D.L., Meeuwig, J.T., and Vincent, A.C.J., 2002. Freshwater protected areas: Strategies for conservation. *Conservation Biology*, **16**, 30-41.

Schneider, R.F., and Meyer, A., 2017. How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations. *Molecular Ecology*, **26**, 330-350.

Schumer, M., Powell, D.L., Delclos, P.J., Squire, M., Cui, R.F., Andolfatto, P., and Rosenthal, G.G., 2017. Assortative mating and persistent reproductive isolation in hybrids. *Proceedings of the National Academy of Sciences of the United States of America,* **114**.

Scribner, K.M., Page, K.S., and Bartron, M.L., 2001. Hybridization in freshwater fishes: A review of case studies and cytonuclear methods of biological inference. *Reviews in Fish Biology and Fisheries*, **10**, 293-323.

Seehausen, O., van Alphen, J.J.M., and Witte, F., 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, **277**, 1808-1811.

Seehausen, O., 2004. Hybridization and adaptive radiation. *Trends in Ecology and Evolution*, **19,** 198-207.

Shechonge, A., Ngatunga, B.P., Bradbeer, S.J., Day, J.J., Freer, J.J., Ford, A.G.P., Kihedu, J., Richmond, T., Mzighani, S., Smith, A.M., Sweke, E.A., Tamatamah, R., Tyers, A.M., Turner, G.F., and Genner, M.J., 2018. Widespread colonization of Tanzanian catchments by introduced *Oreochromis* tilapia fishes: the legacy from decades of deliberate introduction. *Hydryobiologia*. Available at: https://doi.org/10.1007/s10750-018-3597-9 [Accessed April 2018].

Shelton, J.M., Samways, M.J., and Day, J.A., 2015. Predatory impact of non-native rainbow trout on endemic fish populations in headwater streams in the Cape Floristic Region of South Africa. *Biological Invasions*, **17**, 365-379.

Shelton, J., Weyl, O., Van Der Walt, J., Marr, S., Impson, D., Maciejewski, K., Tye, D., Dallas, H., Esler, K., 2017. Effect of an intensive mechanical removal effort on a population of non-native rainbow trout *Oncorhynchus mykiss* in a South African headwater stream. *Aquatic Conservation-Marine and Freshwater Ecosystems*, **27**, 1051-1055.

Shukla, R., and Bhat, A., 2017. Morphological divergences and ecological correlates among wild populations of zebrafish (*Danio rerio*). *Environmental Biology of Fishes*, **100**, 251-264.

Skelton, P.H., and Davies, M.T.T., 1986. Trout in South Africa. *Ichthos Special Edition* **1,** 1–20.

Snoeks, J., Hanssens, M., Ngatunga, B., Chisambo, J., Nyasulu, T., 2004. *Identification, distribution and taxonomy of Malawian cichlids: a look to the future*. In: M.J. Snoeks, ed. 2004. The cichlid diversity of Lake Malawi/Nyasa.Niassa: identification, distribution and taxonomy. 354-356.

Soler, L., Conte, M.A., Katagiri, T., Howe, A.E., Lee, B., Amemiya, C., Stuart, A., Dossat, C., Poulain, J., Johnson, J., DiPalma, F., Lindblad-Toh, K., Baroiller, J., D'Cotta, H., Ozouf-Costaz, C., and Kocher, T.D., 2010. Comparative physical maps derived from BAC end sequences of tilapia (*Oreochromis niloticus*). *BMC Genomics*, **11**, 636.

Stebbins, G.L., 1959. The role of hybridization in evolution. *Proceedings of the American Philosophical Society*, **103**, Commemoration of the Centennial of the Publication of "*The Origin of Species*" by Charles Darwin, 231-251. Available at: http://www.jstor.org/stable/985151. [Accessed October 2016].

Strayer, D.L., and Dudgeon, D., 2010. Freshwater biodiversity conservation: Recent progress and future challenges. *Journal of the North American Benthological Society*, **29**, 344-358.

Taylor, M.D., Chick, R.C., Lorenzen, K., Agnalt, A., Leber, K.M., Blankenship, H.L., Haegen, G.V., and Loneragan, N.R., 2017. Fisheries enhancement and restoration in a changing world. *Fisheries Research*, **186**, 407-412.

Todesco, M., Pascual, M.A., Owens, G.L., Ostevik, K.L., Moyers, B.T., Hübner, S., Heredia, S.M., Hahn, M.A., Caseys, C., Bock, D.G., and Rieseberg, L.H., 2016. Hybridization and extinction. *Evolutionary Applications*, **9**, 892-908.

Trewavas, E., 1983. *Tilapiine fishes of the genera* Sarotherodon, Oreochromis *and* Danakilia. British Museum (Natural History), London. UK.

Turpie, J.K., Forsythe, K.J., Knowles, A., Blignaut, J., and Letley, G., 2017. Mapping and valuation of South Africa's ecosystem services: A local perspective. *Ecosystem Services*, **27**, 179-192.

Walsh, J., Shriver, W.G., Olsen, B.J., O'Brien, K.M., and Kovach, A.I., 2015. Relationship of phenotypic variation and genetic admixture in the Saltmarsh-Nelson's sparrow hybrid zone. *AUK Ornithological Advances*, **132**, 704-716.

Welcomme, R.L., Cowx, I.G., Coates, D., Christophe, B., Funge-Smith, S., Halls, A., Lorenzen, K. 2010. Inland Capture Fisheries. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences.* **365**, 2881-96.

Wilcox, C.L., Motomura, H., Matsunuma, M., and Bowen B.W., 2018. Phylogeography of Lionfishes (*Pterois*) indicate taxonomic ovr splitting and hybrid origin of the invasive Pterois volitans. *Journal of Heredity*, 162-175.

Wiley, C., Qvarnström, A., Andersson, G., Borge, T., and Sætre, G., 2009. Postzygotic isolation over multiple generations of hybrid descendants in a natural hybrid zone: How well do single generation estimates reflect reproductive isolation? *Evolution*, **63**, 1731-1739.

Wolf, D.E., Takebayashi, N., and Rieseberg, L.H., 2001. Predicting the risk of extinction through hybridization. *Conservation Biology*, **15**, 1039-1053.

Wringe, B.F., Stanley, R.R.E., Jeffery, N.W., Anderson, E.C., and Bradbury, I.R., 2017. HYBRIDDETECTIVE: A workflow and package to facilotate the detection of hybridization using genomic data in R. *Molecular Resources*, **17**, 275-284.

Zengeya, T.A., Decru, E. and Vreven, E. 2011. Revalidation of Hepsetus cuvieri (Castelnau, 1861) (Characiformes: Hepsetidae) from the Quanza, Zambezi and southern part of the Congo ichthyofaunal provinces. *Journal of Natural History,* **45**(27), pp. 1723-1744.

Zengeya, T.A., Booth, A.J., and Chimimba, C.T., 2015. Broad niche overlap between invasive Nile tilapia Oreochromis niloticus and indigenous congenerics in Southern Africa: Should we be concerned? *Entropy*, **17**, pp. 4959-4973.

Zengeya, T.A., Ivey, P., Woodford, D.J., Weyl, O., Novoa, A., Shackleton, R., Richardson, D., van Wilgen, B., 2017. Managing conflict-generating invasive species in South Africa: Challenges and trade-offs. *Bothalia African Biodiversity and Conservation,* **47**(2), a2160. Available at: https://doi.org/10.4102/abc.v47i2.2160 [Accessed March 2018].